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Marine *Dendryphiella* species from different geographical locations: an integrated, polyphasic approach to its taxonomy and physioecology

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Dedication

For my grandfather … Anastacio Espinoza

(19.06.1928 – 07.09.2005)

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- to God Almighty, glory, honor and praise are yours, now and forever…

Abstract

The marine *Dendryphiella* species, *D. arenaria* and *D. salina*, are mainly identified based on their conidiogenesis, conidiophore and conidial morphology. The assumed high taxonomic value of these parameters led to the recent inclusion of the marine *Dendryphiella* in the genus *Scolecobasidium*. Thus, according to the currently valid taxonomy, the species are named *S. arenarium* and *S. salinum* with *D. arenaria* and *D. salina* as the respective synonyms. However, due to significant dissimilarity in the physioecological and other morphological characters, there is still disagreement on the placement of these species within *Scolecobasidium*. This research paper, therefore, aimed to determine the position of the marine *Dendryphiella* species with respect to the modern molecular phylogeny of ascomycetes. Furthermore, it aimed to determine to what extent *D. arenaria* and *D. salina* can be differentiated from each other on the basis of their genetic and phenotypic analysis.

Dendryphiella strains were isolated from various substrates collected along coastal areas in subtropical (Gulf of Mexico) and temperate (North Sea, Baltic Sea, Mediterranean Sea, English Channel) waters. Genomic DNA was extracted from strains of both marine and terrestrial *Dendryphiella* and from 10 representative strains of 7 *Scolecobasidium* species. RAPD profiles were initially considered in grouping the isolates for subsequent gene sequencing. Analysis of the partial *rpb*2 sequences was used to find the next closest taxonomic relatives, while the infragenic structure of marine *Dendryphiella* was detected by the variable ITS 1 and 2 of the rDNA repeat

and the large introns of *tef*1 gene. Production of enzymes using cultural methods and API ZYM assay, as well as BIOLOG Phenotype MicroArrays (PM) were used to assess the ability of the *Dendryphiella* strains to utilize different substrata. Secondary metabolic profiles of their crude culture extracts were also detected by TLC and HPLC-DAD. Physiological responses to abiotic and biotic factors as well as their antimicrobial activities were also studied on the different *D. arenaria* and *D. salina* strains.

Sequence analysis of the ITS 1 and 2, *tef*1 and *rpb*2 genes showed that the marine *Dendryphiella* strains formed two sister clades, which correspond to *D. arenaria* and *D. salina*. Both species belong to the family *Pleosporaceae*, with *Pleospora* spp. (anamorph *Stemphylium* spp.) as the next taxonomic relative. All *Scolecobasidium* species sequenced formed a distinct genetically isolated phylogenetic group outside of the class *Loculoascomycetes*, and thus, are not genetically related to *Dendryphiella*. The *Dendryphiella* species from different geographical locations exhibited similar enzyme and secondary metabolic profiles, but differed significantly in their carbon utilization profiles which can be used to discriminate not only the two species, but also sub-populations of *D. arenaria* and *D. salina*. All tested strains grew on the different investigated parameters demonstrating phenotypic plasticity, but optimally on culture medium with added marine salts, at pH values between 6.5 – 8 and at an incubation temperature of 25 ^{o}C. The culture extracts were antimicrobial, though production of the biologically active metabolites was strain specific.

Abstrakt

Die Identifizierung der marinen *Dendryphiella*-Spezies, *D. arenaria* und *D. salina,* basierte hauptsächlich auf der Konidiengenesis und der Morphologie ihrer Konidiophoren und Konidien. Der hohe taxonomische Wert dieser Parameter führte zu der heutigen Annahme, die marine *Dendryphiella* gehöre der Gattung *Scolecobasidium* an. Aufgrund der gegenwärtig geltenden Taxonomie enthielten die beiden Spezies die Namen *S. arenarium* und *S. salinum*, sowie die Synonyme *D. arenaria* und *D. salina*. Anderseits führten signifikante Diskrepanzen in der Physioökologie der beiden Gattungen sowie Unterschiede anderer morphologischer Merkmale zu einer Unstimmigkeit, die die Position der beiden Arten innerhalb der Gattung *Scolecobasidium* in Frage stellte. Das Ziel der vorliegenden Dissertation war den Status der untersuchten Spezies im Hinblick auf die moderne Phylogenie von Ascomyceten zu bestimmen. Darüber hinaus sollte geklärt werden, inwieweit eine Differenzierung zwischen den beiden Spezies mittels genetischer und phenotypischer Analyse möglich ist.

Die *Dendryphiella*–Stämme wurden aus subtropischen Küstengebieten (Golf von Mexico) sowie aus den Gewässern der gemäßigten Zone (Nordsee, Ostsee, Mittelmeer und English Channel, UK) isoliert. Zur Extraktion der genomischen DNA wurden marine und terrestrische *Dendryphiella*-Isolate sowie 10 repräsentative Stämme von 7 *Scolecobasidium* - Spezies herangezogen. Die Eingruppierung der Isolate für die Gensequenzierung erfolgte mittels RAPD-Profile. Die Analyse der partiellen *rpb2*-Sequenz wurde zur Ermittlung von taxonomisch

nahverwandten Spezies herangezogen. Die Aufklärung der infragenische Struktur von den *Dendryphiella*-Arten erfolgte durch die Bestimmung der variablen repeat-Regionen von ITS 1 und 2 sowie des Introns von partiellem *tef1*-Gen. Die Untersuchung der Enzymproduktion und der daraus resultierenden Fähigkeit von den *Dendryphiella*-Stämmen zur Verstoffwechslung unterschiedlicher Substrate, erfolgte unter Anwendung konventioneller Kultivierungsmethoden, API ZYM Assay sowie BIOLOG Phenotype MicroArrays (PM). Die Profile der aus den Kulturrohextrakten gewonnen Sekundärmetabolite wurden mittels TLC und HPLC-DAD bestimmt. Verschiedene *D. arenaria*- und *D. salina* - Stämme wurden auf ihre physiologische Antwort auf den biotischen und abiotischen Faktoren sowie auf antimikrobielle Aktivitäten hin untersucht.

Die Sequenzanalyse der ITS 1 und 2, *tef1* und *rpb*2-Gene ergab, dass die marinen *Dendryphiella* – Stämme zwei Gruppen formen, die zu *D. salina* und *D. arenaria* korrespondierten. Beide Spezies gehören zu Familie *Pleosporaceae*. Die nächstverwandte taxonomisch relevante Art bildet *Pleospora* spp. (Anamorph – *Stemphylium* spp.). Alle sequenzierten *Scolecobasidium*–Spezies bilden eine eindeutig isolierte phylogenetische Gruppe, die außerhalb der *Loculoascomycetes*-Klasse einzuordnen ist. Somit ist keine genetische Verwandtschaft zu *Dendryphiella*-Spezies nachzuweisen. Die von verschiedenen geographischen Regionen isolierten *Dendryphiella*-Arten zeigen ähnliche Enzym- und Metabolitprofile. Sie unterscheiden sich jedoch signifikant in Substratverwertung, was eine Differenzierung nicht nur von den beiden untersuchten Spezies, sondern

auch Subpopulationen der *D. arenaria* und *D. salina* ermöglicht. Das Wachstum der Stämme unter den diversen Bedingungen weist auf ihre phänotypische Plastizität hin. Als optimal erweist sich für die Pilzisolate ein mit Meeressalz supplementiertes Kultivierungsmedium, der pH-Wert von 6.5 – 8 sowie eine Inkubationstemperatur von 25 °C. Die Kulturextrakte zeigen antimikrobielle Eigenschaften; die Produktion von biologisch aktiven Metaboliten ist jedoch stammspezifisch.

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I. Introduction

A. On the definition, diversity and role of marine fungi

The earliest known definition of marine fungi was provided by Kohlmeyer (1974). He described an obligate marine fungi as those that grow and sporulate exclusively in estuarine or marine habitats. However, fungal species from terrestrial or freshwater areas able to grow in estuarine or marine environments were defined as facultative marine fungi. Hyde *et al*. (2000) further defined marine fungi as not a taxonomically, but rather an ecologically and physiologically defined group. Thus, known terrestrial fungal genera often have one or few species of marine origin.

Of the 74,000 fungal species currently described (Hawksworth *et al*., 1995; Hawksworth, 2001), only less than 1 % were identified as marine fungi. Through the years, the number of recognized marine taxa increased from 321 (Kohlmeyer & Volkmann-Kohlmeyer, 1991) to 444 and included 360 species in 177 genera belonging to the Ascomycotina, 10 species in 7 genera belonging to the Basidiomycotina and 74 species in 51 genera belonging to mitosporic fungi (Deuteromycotina) (Hyde *et al*., 2000), a reflection of the growing interest on these ecologically important microorganisms. However, only one taxonomic order, the Halosphaeriales, had fungi mostly of marine origin (Jones, 1995), while others remained part of or classified with known terrestrial genera. This is not suprising as phylogenetic analysis supported the possible terrestrial origins of marine fungi, e.g. the marine ascomycetes belonging to *Halosphaeriales* showed two

lineages which were both independently derived from terrestrial ancestors (Spatafora *et al*., 1998).

The biodiversity of marine fungi can be influenced by various factors including availability and nature of substrata, competition between fungi and other physicochemical factors (Jones, 2000). Their geographical distribution, however, remains controlled mainly by temperature (Hughes, 1974; Kohlmeyer, 1983), though, microfungal communities, for example in mangroves, were more similar within a single ocean basin than between different ocean basins (Schmit & Shearer, 2004).

Marine fungi play an important role in the marine ecosystems (Hyde *et al*., 1998). They are major decomposers of woody and herbaceous substrata as well as dead animals and animals parts along shores and in seas and oceans. As saprophytes, they are directly involved in nutrient cycling in aquatic habitats and along coastal shores and intertidal zones. Marine fungi may also be important pathogens of plants, algae and animals or may enter into a symbiotic relationship with other microorganisms in the form of lichens, mycophycobioses or mycorrhizas in salt-marsh plants.

Fungi from the marine habitats have also been tested for their application in bioremediation, e.g. in degradation of hydrocarbons (Kirk & Gordon, 1988) , in bioaccumulation of the herbicide atrazine (Schocken *et al*., 1982; Schocken & Speedie, 1982a, 1982b) and in decolourization of synthetic dyes and beach plant effluents (Raghukumar, 2000). Production of degradative enzymes by these microorganisms was also reported (Gessner, 1980; Torzilli, 1982; Molitoris & Schaumann, 1986; Rohrmann & Molitoris,

1992) and opened possibilities for their biotechnological application. Marine fungi are also known to be prolific source of biologically active natural products (Liberra & Lindequist, 1995; König & Wright, 1996).

B. The marine *Dendryphiella* species : *D. arenaria* and *D. salina*

B.1. Geographic distribution and economic importance of *Dendryphiella* spp.

Among the most commonly isolated marine fungi are species belonging to the genus *Dendryphiella*. Of the 12 species currently listed under this genus, two are known to be of marine origin, *Dendryphiella arenaria* Nicot (= *Scolecobasidium arenarium* Ellis) and *D. salina* (Sutherland) Pugh et Nicot (= *S. salinum* Ellis). These hyphomycetes are described as saprobic, cosmopolitan, marine fungi, having been isolated from soil samples in salt marshes (Pugh, 1962a, 1962b; Pugh & Beeftink, 1980) or from living or decaying seaweeds (Miller & Whitney, 1981; Genilloud *et al*., 1994), seagrasses (Newell & Fell, 1980; Newell, 1981), driftwoods and submerged woods (Jones, 1962; Jones & Oliver, 1964; Byrne & Jones, 1974; Kirk & Brandt, 1980; Strongman *et al*., 1985) collected along coastal areas and intertidal zones in tropical, sub-tropical and temperate waters (Fig. 1). Such worldwide occurrence renders these species ideal model systems to study ecophysical and morphophysiological adaptation of marine fungi to salinity (Clipson & Jennings, 1992; Edwards *et al*., 1998). Culture extracts of strains of marine *Dendryphiella* have also been reported to exhibit anti-angiogenesis (Otsuka *et al*., 1992) and antimicrobial activities (dela Cruz *et al*., 2006), though biological activities appeared strain-specific. Various

enzymes with potential industrial application produced by these organisms have been patented (Hunter *et al*., 1987; Pedersen *et al*., 1995; Halkier *et al*, 2000) so was its ability to produce xylitol as a recombinant host (Harkki *et al*., 2004).

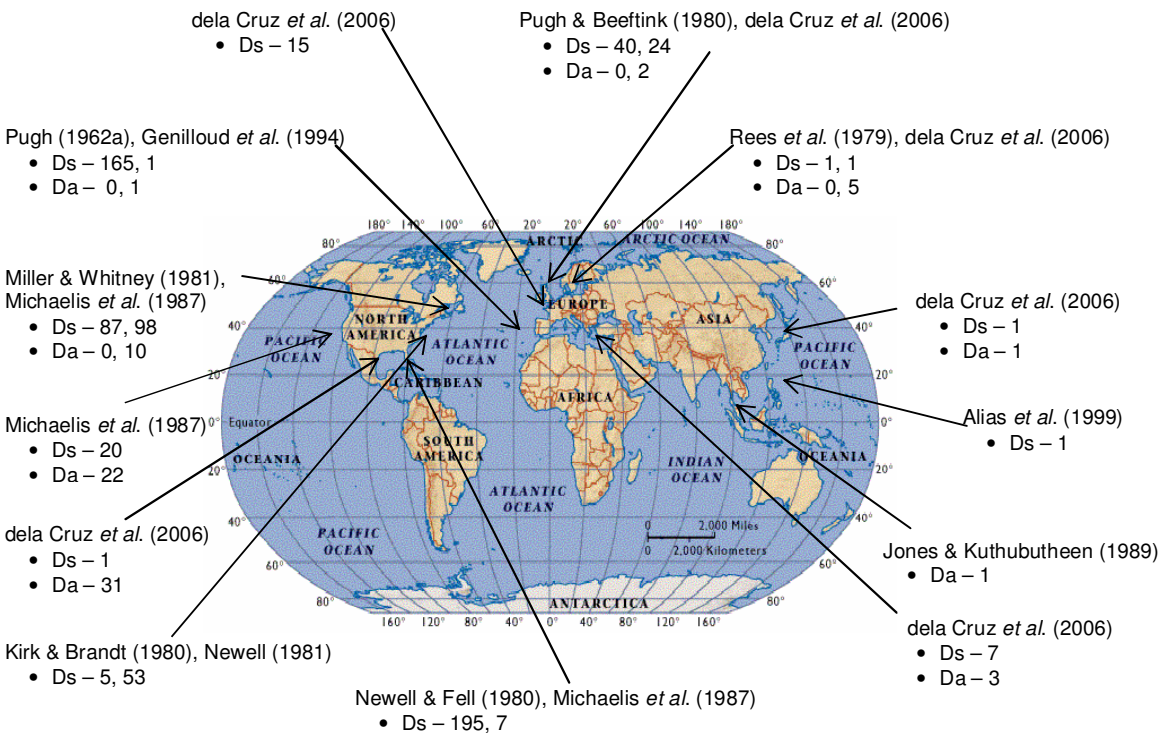


Figure 1. Worldwide geographic distribution of *D. arenaria* (Da) and *D. salina* (Ds). Strains were isolated from various substrates, e.g. sand, seafoams, marine sediments, living or decaying algae and plant materials. See Literature Cited for the complete bibliographic entry.

B.2. Taxonomy of *D. arenaria* and *D. salina*

Sutherland (1916) was first to isolate and identify the marine species of the genus *Dendryphiella* as saprophytic on various seaweeds. Initially named as *Cercospora salina*, it was described to have hyaline or slightly coloured mycelium with erect or sub-erect, simple or branched conidiophores that had terminal or sub-terminal swellings from which olive-green or brown conidia arise. The conidia vary and can be of short, broad forms (30 – 45 μm x 8 – 10 μm) with 3 – 5 septate or of long types (50 – 75 μm x 6 – 9 μm) with 5 – 9 septa (Fig. 2). More than forty years later, Nicot (1958) isolated an arenicolous marine fungus with subhyaline to pale brown mycelium and conidiophores that are large at base. Their conidia varied in form and size (10 – 15 μm x 3.5 – 5.5 μm and 13 – 20 μm x 4.5 – 6.5 μm) with 1 – 3 septate. The species was later identified as *Dendryphiella arenaria* (Fig. 2).

Pugh & Nicot (1964) studied the taxonomic position of *C. salina* and compared it with *D. arenaria*. Both species possessed many similar features ecologically, physiologically and morphologically, and thus, were described as congeneric. As a consequence, *C. salina* was then renamed *Dendryphiella salina* and the morphology of the diffuse conidiophore has been adopted as the primary character in their taxonomy.

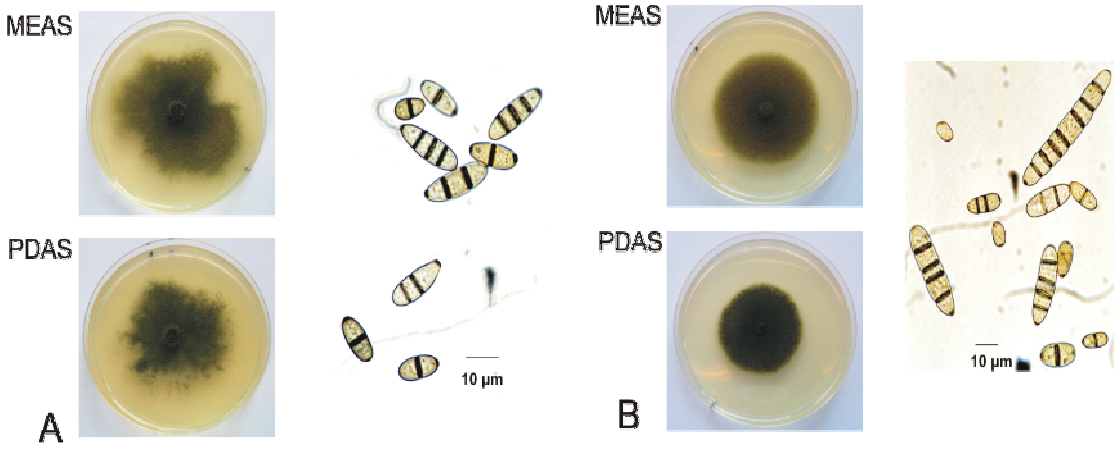


Figure 2. Colony appearance and conidial morphology of *D. arenaria* (A) and *D. salina* (B). Strains were grown on Malt Extract Agar (MEAS) and Potato Dextrose Agar (PDAS) supplemented with 33 g L^{-1} marine salts and incubated at 25 °C for 5 days. Conidia were obtained from strains cultured on Potato Carrot Agar (PCAS) supplemented with 33 g L^{-1} marine salts and incubated at 25 °C for 14 days.

Differences in the length of the conidiophores, the size of the conidia and the degree of septation have often been used to differentiate the two marine species of *Dendryphiella*. However, conidial morphology can be affected by conditions of culture, e.g. *D. arenaria* appeared to produce smooth-walled conidia on saline media (Pugh & Nicot, 1964), though this assertion was not supported by the recent comparative morphological study of *D. salina* and *D. arenaria* (Edwards *et al.*, 1998). Identification keys on these marine fungi also showed overlapping characters which led to difficulties and uncertainties in their identification (Table 1). Michaelis *et al.* (1987) also observed such overlapping characters in their population studies of *D. arenaria* (31 strains) and *D. salina* (127 strains) and which led them to question whether two marine species of *Dendryphiella* or a single species with morphological variations exist. Though the variability observed in their

isozyme electrophoretic data was very low compared to that differentiating other fungal genera, the existence of two species was still concluded. This led them to employ other methods such as ELISA to differentiate the marine species of *Dendryphiella*; the method was found to be sensitive in detecting anti-*D. arenaria* and anti-*D. salina* antibodies (Mohamad *et al*., 1989).

Table 1. Differences in conidial morphology of *D. arenaria* and *D. salina* based on published identification keys.

		Pugh & Nicot (1964)	**Ellis (1976)**	**Kohlmeyer & Kohlmeyer (1979)**
D. arenaria				
conidiophores		15 – 25 µm long or much longer (80 – 90 µm)	up to 90 µm long	15 – 90 µm long
conidia	length	13 – 20 µm (10 – 15 µm)	8 – 25 µm	9 – 20 µm
	width	4.5 – 6.5 µm (3.5 – 5.5 µm)	5 – 7 µm	3.5 – 6.5 µm
	septa	predominantly 3 (1-septate)	mostly 1 – 3, rarely with 4 or 5	1 – 3 -septate
D. salina				
conidiophores		15 – 60 µm long	up to 40 µm long	15 – 60 µm long
conidia	length	20 – 45 µm (45 – 70 µm)	16 – 65 µm	14.5 – 75 µm
	width	6 – 9 µm (6 – 9 µm)	5 – 9 µm	5.5 – 10.5 µm
	septa	predominantly 3 – 5 (5 – 7)	2 – 7 (mostly 5)	1 – 9 (-11) - septate

Other ambiguities to the taxonomic status of *D. arenaria* and *D. salina* occurred when Ellis (1976) moved, without providing explanations, the two species to the genus *Scolecobasidium* and named them as *S. arenarium* and *S. salinum* with *D. arenaria* and *D. salina* as their respective synonyms, though species of the two genera differed both morphologically and physiologically (Fig. 3). At present, these names are listed as the valid taxonomical names for these species. Domsch *et al.* (1980) accepted this change in nomenclature and argued that *D. salina* is not congeneric with *D. vinosa* as the conidiophores were pale brown, little differentiated and with their blastoconidia abstricted from narrow denticles. But, they also mentioned that the species' faster growing colonies and large conidia are unusual for the genus *Scolecobasidium*. However, many marine mycologists do not agree with this classification and still listed the two species as *D. arenaria* and *D. salina*, e.g. in the published guides to filamentous higher marine fungi (Kohlmeyer & Kohlmeyer, 1979; Kohlmeyer & Volkmann-Kohlmeyer, 1991; Hyde *et al*., 2000). Kohlmeyer and Kohlmeyer (1979) further argued that the conidia of *D. arenaria* and *D. salina* have enteroblastic origin and lack denticles as opposed to the polyblastic, denticulate conidiogenous cells with long, narrow-cylindrical, thread-like denticles in *Scolecobasidium* as previously described by Ellis (1971).

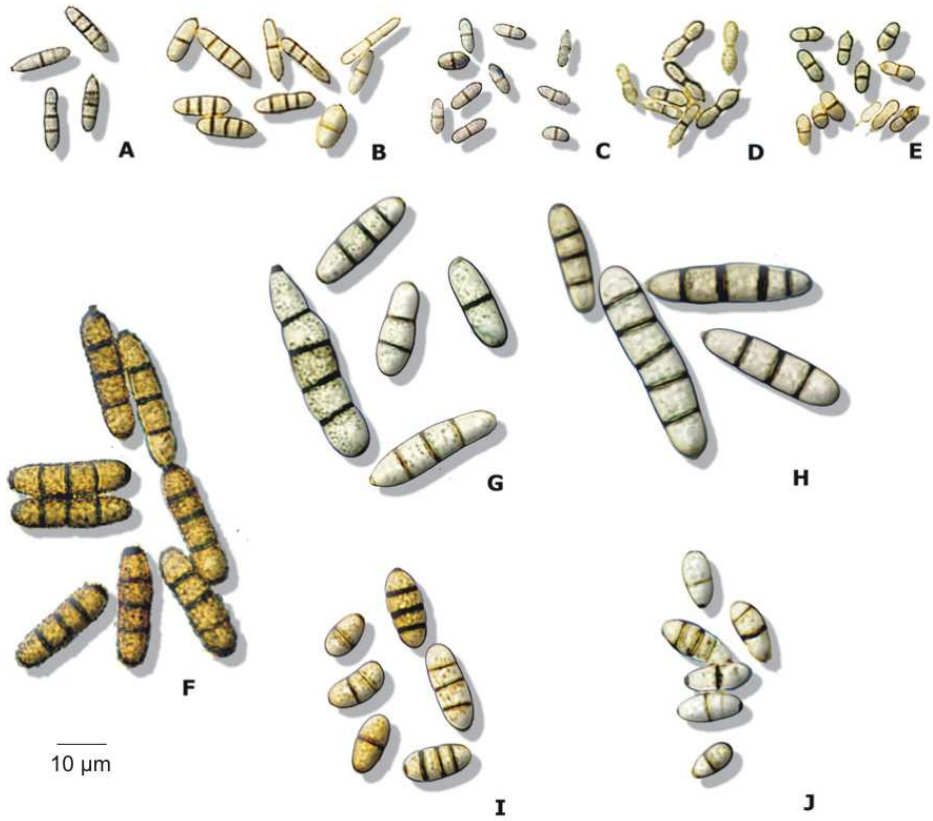


Figure 3. Conidial morphology of *Scolecobasidium* and *Dendryphiella* species. *Scolecobasidium* was represented by *S. variabile* (A, B), *S. humicola* (C) and *S. constrictum* (D, E). The terrestrial *Dendryphiella vinosa* (F) and the marine species *D. salina* (G, H) and *D. arenaria* (I, J) were also shown. Strains were grown on Potato Carrot Agar (PCA) with and without 33 g L^{-1} marine salts at 25 ^{o}C for 14 days.

B.3. Physiological studies on *D. arenaria* and *D. salina*

Earlier studies on *D. arenaria* and *D. salina* focused on the effects of abiotic factors such as salinity, temperature, pH and light or darkness on mycelial growth and spore germination. *Dendryphiella salina* and many other marine fungi do not require seawater, and their growth was found to be somewhat better at lower salinities (Jones & Jennings, 1964). Lorenz & Molitoris (1992) reported that isolates of *D. salina* exhibited the "*Phoma-*

pattern of growth", meaning that at higher temperatures the salinity optimum increases (Richie, 1957). Both growth and spore germination have been found to be optimal at 20 - 30 °C, pH-values between 5 and 7, both with and without light (Curran, 1980; Duffy *et al*., 1991; Panebianco, 1994).

The ubiquity of these fungi in different marine waters had also led to several *in vitro* studies emphasizing their potential to utilize or degrade organic compounds common to the range of substrata from which they have been isolated. *D. salina* and *D. arenaria* have been found to synthesize numerous degradative enzymes, e.g. cellulase by both *D. salina* (Gessner, 1980; Rohrmann & Molitoris, 1992) and *D. arenaria* (MacDonald & Speedie, 1982), and amylase, lipase, β-glucosidase and xylanase, but not the lignin-degradative tyrosinase and laccase by *D. salina* (Gessner, 1980). *D. salina* were also capable of degrading hydrocarbons, e.g. hexadecane, 1-hexadecene, pristane, tetradecane (Kirk & Gordon, 1988) and the herbicide atrazine (Schocken *et al*., 1982; Schocken & Speedie, 1982a, 1982b), providing evidence for their potential use in bioremediation. Degradation of algal components has also been explored, suggesting that these fungi are involved in actual breakdown of, and not just as mere epiphytes on, seaweeds. *D. salina* can synthesize both extracellular laminarinase (Grant & Rhodes, 1992) and poly-β-D-1,4-mannuronide lyase (Shimokawa *et al*., 1997), an extracellular enzyme hydrolytic of alginate in brown algae.

Organisms may produce secondary metabolites in response to their environments, e.g. to protect themselves from competitors. Thus, metabolite production may vary with habitat. Marine *Dendryphiella* species have been

found to produce dendryphiellin A – G, dendryphiellic acid A – B, glyceryl dendryphiellate A, dendryphiellin A1, and dendryphiellin E1 and E2 from cultures of *D. salina* (Guerriero *et al*., 1988, 1989, 1990). Biological activities of the metabolites were not reported. However, in a dual-culture experiment, *D. salina* showed no antagonism against *Gliocladium roseum*, and, hence, was described as a poor competitive saprophyte (Pugh, 1974). Otsuka *et al*. (1992) also reported the absence of antimicrobial activities against several test microorganisms of culture extract of *D. arenaria*, in their paper described as *Scolecobasidium arenarium*, but showed anti-angiogenesis activities. To our knowledge, no further studies have been conducted with respect to the biological activities of the secondary metabolites of the *Dendryphiella s*pecies.

C. Statement of the Problem and Objectives of the Study

The ecological importance and potential biotechnological application of *D. arenaria* and *D. salina* merits a thorough study of these fungi. However, some basic questions on the taxonomy and ecophysiology of these microorganisms remain unanswered. Also, much of our knowledge on these ecologically important marine hyphomycetes came from studies of either a single species or few strains. Thus, extensive comparative investigations were done on strains isolated on a global scale in this research paper.

Due to taxonomic ambiguities of these marine species, this reseach paper aims to answer the following questions:

1. To which genus do marine *Dendryphiella* species belong?
2. To what extent do *D. arenaria* and *D. salina* differ from each other?

In order to answer these problems, this dissertation specifically aims:

1. to isolate, purifiy and characterize strains of *D. arenaria* and *D.salina* from various substrates collected from different geographical locations and climatic zones,
2. to extract their genomic DNA, amplify and sequence their genes encoding for the Internal Transcribed Spacer region of the rDNA (ITS 1 and 2), the translation elongation factor (*tef*1) and RNA polymerase II subunit (*rpb*2), and analyse the gene sequences between the two species as well those of the representative strains of terrestrial *Dendryphiella* and *Scolecobasidium* species,
3. to determine the similarities and differences in the metabolic profiles (extracellular enzymes, carbon source utilization, secondary metabolites) of the two *Dendryphiella* species,
4. to determine physiological and adaptive growth responses of *D. arenaria* and *D. salina* to several abiotic and biotic factors present in its respective ecological niches, and
5. to assess and evaluate the culture crude extracts of selected strains of *Dendryphiella* for their biological activities and potential source of novel bioactive secondary metabolites

However, this research paper does not aim to purify, identify and elucidate the structure of the bioactive secondary metabolites nor investigate their metabolic activities at the cellular, microscopic level.

II. Materials and Methods

A. Isolation and identification of *Dendryphiella* strains

Living and decaying algal and plant materials were collected along the intertidal zones from different geographical locations, i.e. Baltic (Bs) and North (Ns) Seas in Germany, Gozo, Malta and Crete, Greece on Mediterranean Sea (Ms), Lulworth Coast in the United Kingdom (Uk), Atlantic Coast of France (Fr), and the Gulf of Mexico (Gm) in Florida, USA. The collected samples were either washed with sterile distilled water or surface-sterilized with 70% EtOH for 1 – 2 seconds or 30 seconds followed by rinsing three times with sterile distilled water. The plant or algal materials were then cut with a flame-sterilized forcep into 5 - 10 mm^2 explants, placed on Potato Carrot Agar (PCA: 20 g L^{-1} cooked and mashed potatoes, 20 g L^{-1} cooked and mashed carrots, 15 g L^{-1} agar; Höller *et al*., 2000) supplemented with 33 g L^{-1} artificial marine salts (Meersalz, Wiegandt GmbH, Krefeld, Germany) and incubated at room temperature (22 – 25 ^{o}C). Fungal colonies growing out of the explants were reisolated and purified by subculture on fresh PCA medium. Then, spore suspensions from isolated strains were serially diluted and streaked on fresh PCA plates. Colonies arising from single spore were subcultured and grown as monospore cultures. Terrestrial species of *Scolecobasidium* and *Dendryphiella* included in the study, generously provided by Dr. Akira Nakagiri (NBRC, Japan), were also serially diluted, streaked on fresh PCA plates and grown as monospore cultures.

Identification of the isolated *Dendryphiella* strains was done following comparison of their conidial morphology with the descriptions provided by

Kohlmeyer & Volkmann-Kohlmeyer (1991) and Ellis (1976). Of the total marine *Dendryphiella* isolates, fifty five strains were initially grown on PCA medium with 33 g L^{-1} marine salts at 25 ^{o}C for 14 days (Table 2). Following incubation, the plates were flooded with 10 ml sterile distilled water, spores dislodged with an inoculating loop and 10 µl of spore suspension placed on a glass slide. Under the brightfield compound microscope (Zeiss, Germany; 1000x), 100 spores per strain were measured individually for its length, width and number of septa.

To study their growth on complex culture media, the *Dendryphiella* strains were initially grown on basal medium containing 10 g L^{-1} glucose, 1 g L^{-1} peptone, 0.1 g L^{-1} yeast extract, 15 g L^{-1} agar and 33 g L^{-1} marine salts (pH 6.5) at 25 ^{o}C for 7 days. After incubation, 5 mm diameter agar blocks were cut approximately 5 mm away from the colony margin with a flame-sterilized cork borer, transferred to the centers of petri dishes pre-filled with Malt Extract Agar (MEAS: per liter 30 g malt extract, 3 g peptone from soyameal, 15 g agar) and Potato Dextrose Agar (PDAS, Merck, Darmstadt, Germany) supplemented with 33 g L^{-1} marine salts; the culture plates were incubated at 25 ^{o}C in the dark. Colony diameter of the resulting growth was measured with a Vernier caliper (three readings per plate), and mean colony extension rate (mcer) was computed as follows:

$$\text{mcer} = \frac{\text{mean colony diameter (day 5)} - \text{mean colony diameter (day 3)}}{\text{number of days of incubation (2 days)}}$$

The morphocultural data were analyzed statistically (ANOVA) with SigmaStat 3.1 (Systat Software Inc., USA) to determine their significant differences.

Table 2. Origin of *Dendryphiella* and *Scolecobasidium* species used in this study.

Strain		GeneBank Accession Nr.			substrate	geographic origin
		ITS1/ITS2	*tef*1	*rpb*2		
D. arenaria						
Bs 01	TUBs 7888 (NBRC 100653)				*Ceramium* sp.	Rügen Island, Germany (Baltic Sea)
Bs 02	**TUBs 7889 (NBRC 100654)**	DQ307290			*Fucus* sp. (dried sample)	
Bs 03	TUBs 7890				*Fucus* sp. (dried sample)	
Bs 04	**TUBs 7891 (UAMH 10474)**	DQ307291	DQ307335	DQ307357	*Polysiphonia urceolata* (Lighf. In Dillw.) Grev.	Friedrichsort, Germany (Baltic Sea)
Bs 94	**TUBs 8520**				sand	Copenhagen, Denmark (Baltic Sea)
Gm 22	**TUBs 7538**	DQ307296			*Hypnea musciformis* (Wulfen in Jacquin) Lamouroux	John's Pass, FL, USA (Gulf of Mexico)
Gm 23	**TUBs 7527 (NBRC 101141)**	DQ307297	DQ307338		*Digenea simplex* (Wulfen) C. Agardh	
Gm 27	TUBs 7541				*Gracillaria* sp.	
Gm 56	**TUBs 8195**	DQ307308			*Sargassum* sp. (decaying material)	
Gm 58	TUBs 8197	DQ307309			*Sargassum* sp. (decaying material)	
Gm 61	TUBs 8200	DQ307310			*Sargassum* sp. (decaying material)	
Gm 77	TUBs 8216				*Zostera marina* L. (decaying material)	
Gm 79	**TUBs 8218**	DQ307311			*Z. marina* (decaying material)	
Gm 28	TUBs 6551	DQ307300			*Sargassum* sp.	
Gm 24	TUBs 7479	DQ307319		DQ307362	*Gracillaria tikvalriae* McLachlan	Fort de Soto, FL, USA (Gulf of Mexico)
Gm 26	**TUBs 7515**	DQ307299			unknown substrate	
Ms 31	TUBs 7911	DQ307302			unknown substrate	Crete, Greece (Mediterranean Sea)
Ms 36	TUBs 7916	DQ307305			unknown substrate	Gozo, Malta (Mediterranean Sea)

Table 2. cont.

Strain		GeneBank Accession Nr.			substrate	geographic origin
		ITS1/ITS2	*tef*1	*rpb*2		
D. arenaria						
Jp 50	**NBRC 32140**	DQ307321			driftwood	Teshio, Hokkaido, Japan (Sea of Japan)
	CBS 181.58 (type strain)	DQ411539	DQ414250	DQ435065	coastal sand under *Ammophila arenaria* Link.	Gironde, France (European Atlantic Coast)
Fr 54	**NBRC 8359 (*ex*-type strain)**	DQ307318	DQ307346		coastal sand under *A. arenaria*	France
Fr 52	**UAMH 1357**	DQ307320	DQ307364	DQ307366	sand	France
D. salina						
Bs 05	**TUBs 7892 (UAMH 10475)**	DQ307292	DQ307336	DQ307358	*P. urceolata*	Friedrichsort, Germany (Baltic Sea)
Fr 53	**TUBs 8147**	DQ307307	DQ307342	DQ307361	*Enteromorpha intestinalis* (L.) Nees.	France (European Atlantic Coast)
Ms 32	**TUBs 7912 (NBRC 101140)**	DQ307303			unknown substrate	Crete, Greece (Mediterranean Sea)
Ms 33	TUBs 7913				unknown substrate	
Ms 34	TUBs 7914				unknown substrate	
Ms 35	**TUBs 7915**	DQ307304	DQ307340		unknown substrate	Gozo, Malta (Mediterranean Sea)
Ms 37	**TUBs 7917**	DQ307306	DQ307341	DQ307360	unknown substrate	
Ms 38	TUBs 7918 (NBRC 100657)	DQ307324			unknown substrate	
Ns 06	TUBs 7893 (NBRC 100655)				*Laminaria digitata* (Huds.) Lamour (drift samples)	Helgoland, Germany (North Sea)
Ns 08	TUBs 7895				*L. digitata* (drift samples)	

Table 2. cont.

Strain		GeneBank Accession Nr.			substrate	geographic origin
		ITS1/ITS2	*tef*1	*rpb*2		
D. salina						
Ns 10	**TUBs 7897 (UAMH 10476)**	DQ307293			*L. digitata* (drift samples)	Helgoland, Germany (North Sea)
Ns 16	TUBs 7903				*L. digitata* (drift samples)	
Ns 20	TUBs 7907				*L. digitata* (drift samples)	
Ns 11	TUBs 7898 (UAMH 10477)				*L. digitata* (decaying material)	
Ns 13	TUBs 7900				*L. digitata* (decaying material)	
Ns 21	**TUBs 7908**	DQ307295			*L. digitata* (decaying material)	
Ns 17	TUBs 7904	DQ307294	DQ307337	DQ307359	*L. digitata* (decaying material)	
Ns 18	TUBs 7905	DQ307325			*Fucus serratus* L. (on rocks, ashore)	
Ns 19	TUBs 7906 (NBRC 100656)				*F. serratus* (on rocks, ashore)	
Ns 45	TUBs 7925				*F. serratus* (on rocks, ashore)	
Ns 29	**TUBs 7909 (NBRC 101143)**	DQ307301			*Glaux maritima* L.	Cuxhaven, Germany (North Sea)
Ns 30	TUBs 7910				unknown substrate	
Uk 80	TUBs 8219				*Fucus* sp.	Lulworth Coast, United Kingdom
Uk 81	TUBs 8220				*Fucus* sp.	(English Channel)
Uk 82	**TUBs 8221**	DQ307312	DQ307343		*Fucus* sp.	
Uk 83	TUBs 8222				*F. serratus*	
Uk 84	TUBs 8223				*F. serratus*	
Uk 85	TUBs 8224				*F. serratus*	

Table 2. cont.

Strain		GeneBank Accession Nr.			substrate	geographic origin
		ITS1/ITS2	*tef*1	*rpb*2		
D. salina						
Uk 86	TUBs 8225	DQ307323			*F. vesiculosus* L.	Lulworth Coast, United Kingdom (English Channel)
Uk 88	TUBs 8227	DQ307313			*F. vesiculosus*	
Uk 90	**TUBs 8229**	DQ307314			*F. vesiculosus*	
Uk 92	TUBs 8231	DQ307322	DQ307365		*F. vesiculosus*	
Gm 25	TUBs 7508 (NBRC 101142)	DQ307298	DQ307339		*Ceramium* sp.	Fort de Soto, Fl., USA (Gulf of Mexico)
Jp 51	**NBRC 32139**	DQ307317	DQ307345		seafoam	Teradomari, Niigata, Japan (Sea of Japan)
	CBS 142.60	DQ411540	DQ414251	DQ435066	*Spartina* sp.	Southampton, United Kingdom (English Channel)
terrestrial *Dendryphiella*						
NBRC 100153 (*Dendryphiella* sp.)		DQ307315	DQ307344	DQ415433	*Eleocharis kuroguwai* Ohwi.	Kamiiwai, Mishima, Niigata, Japan
NBRC 32669 (*D. vinosa*)		DQ307316		DQ415440	*Enhalus acoroides* (L.) Royle. (decomposing leaf)	Iriomote Is., Okinawa, Japan
***Scolecobasidium* species**						
NBRC 8855 (*S. constrictum*)		DQ307326	DQ307347	DQ415430	unknown substrate	unknown geographic origin
NBRC 9375 (*S. constrictum*)		DQ307327	DQ307348	DQ415431	dead leaf	unknown geographic origin
NBRC 9845 (*S. terreum*)		DQ307328	DQ307349	DQ415432	aquatic sediment	unknown geographic origin
NBRC 30094 (*S. variabile* = *S. tshawytschae*)			DQ307350	DQ415434	soil	unknown geographic origin

Table 2. cont.

Strain	GeneBank Accession Nr.			substrate	geographic origin
	ITS1/ITS2	*tef*1	*rpb*2		
***Scolecobasidium* species**					
NBRC 30095 (*S. verruculosum*)	DQ307329	DQ307351	DQ415435	soil	unknown geographic origin
NBRC 30208 (*S. tricladiatum*)	DQ307330	DQ307352	DQ415436	dead leaf	unknown geographic origin
NBRC 30449 (*S. cateniphorum*)	DQ307331	DQ307353	DQ415437	*Lithocarpus edulis* (Makino.) Nakai. (litter)	unknown geographic origin
NBRC 31974 (*S. humicola*)	DQ307332	DQ307354	DQ307363	soil	Russia
NBRC 32054 (*S. humicola*)	DQ307333	DQ307355	DQ415438	*Eucalyptus* sp. (leaf)	Israel
NBRC 32268 (*S. variabile* = *S. tshawytschae*)	DQ307334	DQ307356	DQ415439	soil	China

[a] Reference strains of *Dendryphiella* and *Scolecobasidium* species were obtained from the National Institute of Technology and Evaluation – Biological Resource Center (NBRC) in Japan, the Centraalbureau voor Schimmelcultures (CBS) in the Netherlands and the University of Alberta Microfungus Collection and Herbarium (UAMH) in Canada. Several strains were deposited in the following culture collections (NBRC and UAMH). The strains were also deposited at the Institute of Microbiology, Technical University Braunschweig (TUBs).

[b] *Dendryphiella* strains from Friedrichsort, Germany, the Mediterranean Sea, Atlantic Coast of France and Lulworth Coast, United Kingdom were isolated by various members of the research groups Aust (TU Braunschweig) and Zeeck (Göttingen University).

[c] Strains in bold letters were used in the production and detection of secondary metabolites.

[d] geographic origin of strains
Baltic Sea (Bs), Atlantic Coast of France (Fr), Gulf of Mexico (Gm), Sea of Japan (Jp)
Mediterranean Sea (Ms), North Sea (Ns), Lulworth Coast, United Kingdom (Uk)

B. Genetic analysis of *Dendryphiella* and *Scolecobasidium* species

B.1. Extraction of genomic DNA

Fifty-seven marine and two terrestrial strains of *Dendryphiella* including two type-strains of *D. arenaria* (NBRC 8359, CBS 181.58) and a reference strain of *D. salina* (CBS 142.60) and 10 representative strains of 7 *Scolecobasidium* species were initially cultured on sterile cellophane-covered 3 % (w v^{-1}) Malt Extract Agar supplemented with 33 g L^{-1} marine salts at 25 ^{o}C in the dark for 3 – 4 days (MEA without salts for terrestrial isolates) (Table 2). Mycelial growth from colony margin, approximately 150 – 200 mg fresh weight, were then scraped-off, ground in liquid nitrogen and the genomic DNA extracted with the DNeasy® Plant MiniKit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Extracted DNA was then stored at -20 ^{o}C until used in the gene sequence analysis.

B.2. RAPD analysis and PCR amplification of nuclear DNA

PCR amplification of the nuclear DNA was done as previously described (Turner *et al.*, 1997; Kullnig-Gradinger *et al.*, 2002; see Appendix B for the PCR parameters). For RAPD analysis, the extracted genomic DNA was PCR-amplified with the primer M13-4fw (5'-AGG CTG TTG GAC ACG GCG GC-3'), purified with the commercially available QIAquick PCR purification Kit (Qiagen GmbH, Hilden, Germany), and the PCR products (20 µl) loaded on 1% agarose gel (300 ml) mixed with 6 µl ethidium bromide in TAE buffer. Gel electrophoresis was set up at 80V for 80 minutes and the DNA bands were visualized with UV and recorded with Gel Doc 2000 (Bio

Rad, USA). The RAPD profiles were then considered in determining the isolates for subsequent gene sequencing.

The gene sequences amplified in this study and the respective primer pairs were: (1) the internal transcribed spacers 1 and 2 (ITS 1 and 2) regions including the flanking 5.8S rRNA gene with the fungal specific primer combinations SR6R (5'-AAG TAG AAG TCG TAA CAA GG-3') and LR1 (5'-GGT TGG TTT CTT TTC CT-3') (White *et al*., 1990), (2) the area covering the large intron of the translation elongation factor 1-alpha gene (*tef*1) with the primer pair EF1-728F (5'-CAT CGA GAA GTT CGA GAA GG-3') (Chaverri & Samuels, 2003) and TEF1-LLErev (5'-AAC TTG CAG GCA ATG TGG-3'), and (3) a portion of the exon between the 5th and 7th eukaryotic conserved amino acid motives of the RNA polymerase II subunit B (*rpb*2) gene with the primers fRPB2-5f (5'-GAY GAY MGW GAT CAY TTY GG-3') and fRPB2-7cr (5'-CCC ATR GCT TGY TTR CCC AT-3') (Liu *et al*., 1999). The partial *rpb*2 sequences was used to find the next closest taxonomic relatives of the marine *Dendryphiella* while their infragenic structure was detected by the variable ITS1 and 2 of the rRNA cluster and the introns of the partial *tef*1 gene. Following amplification, the PCR products were purified with the commercially available QIAquick PCR purification Kit (Qiagen GmbH, Hilden, Germany), dried at 37 ^{o}C and sent for outdoor sequencing at MWG-Biotech AG, Martinsried, Germany. Sequences were then submitted to GenBank and their accession numbers are listed in Table 2.

B.3. Analysis of gene sequences

Initially, related gene sequences were retrieved from GenBank for the phylogenetic analysis. Due to the uncertain phylogenetic position of both the *Dendryphiella* and *Scolecobasidium* species, several strategies were employed to obtain sequences of all possible taxonomic relatives. For the standard Ascomycete phylogenetic marker *rpb*2, the comprehensive list of available sequences was obtained from Liu *et al.* (1999) and Liu & Hall (2004). Every discrete *rpb*2 sequence was likewise subjected to nucleotide – nucleotide similarity search (www.ncbi.nlm.nih.gov/blast) to obtain other related sequences not listed in the published source. For the ITS 1 and 2 and the *tef*1 genes, related sequences were also retrieved following nucleotide similarity search in the GenBank. However, due to the large number of retrieved sequences of similar species, e.g. *Pleospora* spp. (*Stemphyllium* spp.) ITS 1 and 2 sequences, the probability to obtain all closely related species was low and was not always successful. Thus, in addition to the retrieved gene sequences, the NCBI taxonomy browser (www.ncbi.nlm.nih.gov/Taxonomy/taxonomyhome.html) was explored to identify related genus and species under the taxonomic class *Dothideomycetes* and were manually checked in GenBank for the presence of the respective phylogenetic markers. The taxonomic identities of the gene sequences retrieved were given according to their records in GenBank and were not futher checked for their accurate identification. The final alignments included the maximum number of potentially related sequences.

The DNA sequences of the three amplified genes of the strains of *D. arenaria* and *D. salina* were initially aligned automatically using ClustalX v.1.81 (Thompson *et al*., 1997) and then, visually aligned using Genedoc 2.6 (Nicholas & Nicholas, 1997). Sequences of the related taxonomic genera and species obtained from the BLAST search were also aligned with the amplified sequences of *Dendryphiella*. The interleaved NEXUS file was formatted from these sequences using PAUP*4.0b10 and then, manually for the MrBayes v3.0B4 program (Huelsenbeck & Ronquist, 2001). To construct the phylogenetic tree, the Bayesian approach to phylogenetic constructions was conducted with MrBayes v3.0B4 (Rannala & Yang, 1996; Yang & Rannala, 1997). The model of evolution and prior settings for individual loci were estimated as previously described by Druzhinina *et al*. (2004). Metropolis-coupled Markov chain Monte Carlo (MCMCMC) sampling was performed with four incrementally heated chains that were simultaneously run for 1 and 3 millions generations. To check for potentially poor mixing of MCMCMC, each analysis was repeated three times. The convergence of MCMCMC was monitored by examining the value of the marginal likelihood through generations. Convergence of substitution rate and rate heterogeneity model parameters were also checked. Bayesian posterior probabilities (PP) were obtained from the 50% majority rule consenses of trees sampled every 100 generations after removing the first 500 trees using the “burn-in” command. According to the protocol of Leache and Reeder (2002), PP values lower than 0.95 were not considered significant while values below 0.9 were not shown on phylograms and radial trees.

C. Phenotypic analysis of *D. arenaria* and *D. salina*

C.1. Analysis of carbon source assimilation patterns

Carbon source utilization by the marine *Dendryphiella* isolates was examined using the BIOLOG Phenotype Microarrays (PM) as recently adapted for analysis of *Hypocrea*/*Trichoderma* (Druzhinina *et al*., 2006). The monospore *Dendryphiella* cultures were initially grown on 2 % (w v^{-1}) Malt Extract Agar (MEA) supplemented with 33 g L^{-1} marine salts at 25 ^{o}C for 10 days. Inoculum was prepared by rolling a sterile, wetted cotton swab over the conidia bearing colony, suspending it in 16 ml sterile phytagel solution (0.25 % phytagel, 0.03 % Tween 40) in disposable borosilicate test tubes (20 x 150 mm) and the spore density adjusted to 75 ± 2 % transmission at 590 nm wavelength. An aliquot of 90 µL conidial suspension was then dispensed into each well of a pre-sterilized Biolog FF MicroPlate (BIOLOG, Hayward, CA) which contained 95 wells of different pre-filled substrata plus one well with water as control. The inoculated microtiter plates were incubated in the dark at 25 ^{o}C and the mycelial growth (turbidity data) was measured after intervals of 96, 120, 144 and 168 hours as the percent absorbance at 750 nm using a semi-automated Biolog MicroStation^{Tm} system microplate reader.

Utilization of algal cell wall components and algal extracts was also examined on 16 *Dendryphiella* strains (Table 3). Distilled water suspensions of spores from 10-day-old cultures of the selected *Dendryphiella* were centrifuged at 3000 rpm for 3 minutes, washed three times with and resuspended in sterile distilled water, and their concentrations for use in inoculation adjusted to 1.0 – 3.0 x 10^{6} spores mL^{-1}. Aliquots of inoculum (20

µL) were added to sterile 96-well microtiter plates, each containing 200 µl CDM (without sucrose) with 3.3 % NaCl, pH 8.0 and, in 3 wells each, 3.0 % (w v^{-1}) of the following carbon sources: D-glucose, D-galactose, D-fructose, D-sucrose, D-mannose, soluble starch, D-glucuronic acid, L-fucose, alginic acid sodium salt (1 & 3 %), fucoidan, laminarin and aqueous extracts from the algae *Chondrus crispus* and *Laminaria digitata* (30 g algal thalli per liter, boiled for 2 hours and filtered). The control wells contained CDM with 3.3 % NaCl, without added carbon source. Microtiter plates were incubated at 25 °C for 5 days and observed under the microscope (40 x) for mycelial growth indicative of substrate utilization.

C.2. Semi-quantitative assay of constitutive enzymes using API ZYM

Strains of *Dendryphiella* were initially cultured on Czapek Dox Agar (Fluka, Germany) supplemented with 33 g L^{-1} marine salts (pH 6.5) at 25 °C for 10 days. Following incubation, a spore suspension was prepared by flooding the grown fungal cultures with 5 ml sterile distilled water; the spore concentration was adjusted to 5.0 x 10^6 spore ml^{-1}. An aliquot of 65 uL of the suspension was then delivered into the API ZYM cupules (Bio Merieux, France) and incubated at 37 °C for 4 hours as described in the manufacturer's instructions. One drop each of ZYM A (25 g Tris-hydroxymethyl-aminomethane, 11 ml 37 % HCl, 10 g sodium lauryl sulfate, 100 ml H_20) and ZYM B (0.12 g Fast Blue BB, 50 ml methanol, 50 ml dimethylsulfoxide) reagents were added to the cupules, which were placed under white light for 10 minutes; the colour reactions were read and

compared with the colour code provided by the manufacturer. Results were recorded as zero (zero nanomole substrate hydrolysed), 1 (< 20 nanomole substrates hydrolysed), 2 (20 – 40 nanomole substrates hydrolysed) and 3 (> 40 nanomole substrates hydrolysed).

C.3. Production of polymer degrading enzymes

The *Dendryphiella* strains were initially cultured on basal medium (BM) containing 10 g L^{-1} glucose, 1 g L^{-1} peptone, 0.1 g L^{-1} yeast extract, 34 g L^{-1} marine salts and 16 g L^{-1} agar, pH 7.0 at 25 ^{o}C for 7 days in the dark. To test for oxidative enzymes, strains were grown on a similar BM, but with a reduced concentration of glucose (2 g L^{-1}), peptone (0.1 g L^{-1}) and yeast extract (0.01 g L^{-1}). Following incubation, agar blocks were removed with a flame-sterilised cork borer (5 mm in diameter) from the margin of actively growing colonies and inoculated at the center of Petri dishes pre-filled with agar media specific for production of the respective extracellular enzymes. Culture plates were incubated at 25 ^{o}C for up to 2 weeks and the detection of enzymes done with various reagents or observation of colour changes in the media as described for the respective methods.

Cellulolytic and hemi-cellulolytic enzymes. Tests for the degradation of crystalline cellulose (cellulose azure agar) and carboxymethycellulose (CMC agar) were conducted to determine the presence of cellulolytic enzymes as described by Pointing (2000). Production of β-glucosidase was determined to be positive when black halos were formed around fungal colonies after growth on Esculin Iron Agar, while

clearance of the opaque xylan agar indicated xylanase activity (Pointing, 2000).

Oxidative enzymes. An agar well test with syringaldazine was used to detect the presence of peroxidase and laccase (Pointing, 2000). Production of brown pigments on tannic acid agar in tubes detected the presence of polyphenol oxidase (Gessner, 1980).

Other extracellular enzymes. Amylase, lipase and urease were produced at 25 ^{o}C on solid culture media, i.e. starch agar, tween 20 agar, urea agar, respectively, supplemented with 33 g L^{-1} marine salts, and the enzymes detected as previously described by Hankin & Anagnostakis (1975).

C.4. Secondary metabolic profiling using TLC and HPLC

Twenty-four strains of *Dendryphiella* including the two terrestrial strains, *D. vinosa* NBRC 32669 and *Dendryphiella* sp. NBRC 100153 (Table 2), were initially grown at 25 ^{o}C in the dark for 4 weeks on Malt Extract-Peptone-Yeast Extract Agar supplemented with 33 g L^{-1} marine salts (MPY without added marine salts for terrestrial strains; Schulz *et al*., 1995). After incubation, the cultures were lyophilized, ground, extracted with buffered ethylacetate and evaporated *in-vacuo*. Dried crude extracts were then dissolved in 1:1 methanol – acetone (1.5 ml per 3 plates) and stored at 15 ^{o}C until use for assay.

To determine the differences in the secondary metabolic profiles between *D. arenaria* and *D. salina*, aliquots (20 uL) of the crude extracts were spotted on TLC silica gel 60 F_{254} aluminium plates (Merck, Darmstadt,

Germany), run in 4 % MeOH in a dichloromethane solvent system and visualized with various spray reagents [H_2SO_4 in EtOH (general spray reagent), Cer-reagent (for triterpenes), $FeCl_3$ in HCl (for phenolic compounds)]; sprayed TLC plates heated for 10 minutes at 110 ^oC, 3 minutes for triterpenes (120 ^oC) and phenolic compounds (60 ^oC). An aliquot of 900 µL crude extract was also air-dried overnight, the weight determined and later dissolved in DMSO to a final concentration of 10 mg ml^{-1}. The crude extracts were then subjected to High-Performance Liquid Chromatography coupled with a Diode Array Detector (HPLC-DAD) by Dr. Karsten Siems, AnalytiCon Discovery (Potsdam, Germany), the peaks corresponding to the metabolites (between 0 – 22 minutes retention time) visually determined from the chromatogram following comparison with the uninoculated MPYS extract (control) and recorded as 1 (present) and 0 (absent).

C.5. Statistical analysis

Analysis of the Phenotype MicroArray was performed only on the turbidity data sets measured at 750 nm, the values being directly proportional to mycelial density. Cluster analyses of these data were then computed using Statistica 6.0 (Statsoft, USA), while one-way ANOVA's were computed with SigmaStat 3.1 (Systat Software Inc., USA) to determine the significant differences between the substrates utilized by isolates of *D. arenaria* vs. *D. salina*. Principal component analysis was performed on substrates with statistically significant utilization. The similarity coefficient between characteristics of the *Dendryphiella* strains was also computed based on their

extracellular enzyme and substrate utilisation profiles (as multi-state data, simple matching coefficient) and their secondary metabolic profiles (as binary data, Jaccard's coefficient) using the NTSYSpc version 2.2 (Exeter Software, USA). In determining the similarity between species and strains, the simple matching coefficient gives equal value to both the positive and negative matches, while the coefficient of Jaccard was appropriate for the secondary metabolic profiles since negative matches were excluded from the analysis (Sneath & Sokal, 1973). Negative values may not necessarily mean the inability of the organism to produce the metabolites, but could be due to the instrument's detection limit.

Table 3. *Dendryphiella* strains tested for the influence of varied abiotic factors and the environmental conditions at their collection sites.

average salinity [b] (g L^{-1})	annual temperature range (°C) [c]	Location and substrates	Strain Number [a]	Identified as [f]
temperate regions				
Baltic Sea		Rügen Island, Germany		
		Ceramium sp.	TUBs 7888 (NBRC 100653)	*D. arenaria*
< 10 [d]	2 – 17	*Fucus* sp. (dried)	TUBs 7889 (NBRC 100654)	***D. arenaria***
		Friedrichsort, Germany		
		Polysiphonia aureolata	TUBs 7891 (UAMH 10474)	***D. arenaria***
			TUBs 7892 (UAMH 10475)	***D. salina***
North Sea		Helgoland, Germany		
		Laminaria digitata (drift samples)	TUBs 7893 (NBRC 100655)	*D. salina*
			TUBs 7897 (UAMH 10476)	***D. salina***
32 – 33 [e]	6 – 18	*Laminaria digitata* (rotten samples)	TUBs 7898 (UAMH 10477)	*D. salina*
		Laminaria digitata (drift samples)	TUBs 7903	*D. salina*
		Cuxhaven, Germany		
		Glaux maritima	TUBs 7909 (NBRC 101143)	***D. salina***
Mediterranean Sea		Crete, Greece		
		unidentified algal substrate	TUBs 7912 (NBRC 101140)	***D. salina***
			TUBs 7914	*D. salina*
> 37 [c]	14 – 26	Gozo, Malta		
		unidentified algal substrate	TUBs 7916	***D. arenaria***
			TUBs 7918 (NBRC 100657)	***D. salina***
sub-tropical region				
Gulf of Mexico		Fort de Soto Park, Florida		
		Gracillaria tikvalriae	TUBs 7479	***D. arenaria***
36 [c]	22 – 29	*Ceramium* sp.	TUBs 7508 (NBRC 101142)	***D. salina***
		John's Pass, Florida		
		Gracillaria sp.	TUBs 7541	*D. arenaria*

[a] Several isolates were deposited at or obtained from the University of Alberta Microfungus Collection and Herbarium (UAMH) in Canada and NITE Biological Resource Center (NBRC) in Japan. TUBs represents the accession code for the strains deposited in the research group's culture collection at the Technical University Braunschweig, Germany.

[b] Salinity values converted from practical salt units (psu) to grams per liter.

[c] http://www.nodc.noaa.gov/OC5/WOA01F/tsearch.html

[d] http://www.fimr.fi/en/itamerikanta/bsds/559.html

[e] http://www.bsh.de/de/Meeresdaten/Beobachtungen/MURSYS-Umweltreportsystem/Mursys_031/seiten/nosa6_01.jsp

[f] Strains were identified according to conidial morphology. Identity of strains in bold letters was confirmed by gene sequence analysis of *rpb*2, ITS1 and 2 and *tef*1 phylogenetic markers.

D. Physiological Responses of marine *Dendryphiella* species

D.1. Growth responses to abiotic factors

Growth responses of sixteen *Dendryphiella* strains (those listed in Table 3) to abiotic physicochemical factors were determined based on measurements on day 3 and 5 of mean colony diameter in triplicate plate culture on Czapek Dox agar Medium (CDM) with sucrose and sodium nitrate, respectively, as sole carbon and nitrogen sources. Test ranges for the parameters were chosen to include those of the natural habitat (Table 3). Inoculation was by placement of a silica gel bead (≈ 2 – 5 mm in diameter, Merck) previously soaked in a distilled water suspension of 10-day-old spores (10^6 spores ml^{-1}) in sterile 0.01% Tween 80 at the center of a petri dish (9 cm. in diameter) with culture medium. Incubation conditions differed for the different parameters being tested. Colony diameter of the resulting growth was measured with a Vernier caliper (three readings per plate), and mean colony extension rate (mcer) was computed as previously described.

$$\text{mcer} = \frac{\text{mean colony diameter (day 5)} - \text{mean colony diameter (day 3)}}{\text{number of days of incubation (2 days)}}$$

Quantitative data were then analyzed statistically (ANOVA) with SigmaStat 3.1 (Systat Software Inc., USA) to determine significant differences between and within species and strains.

Salinity. To determine the optimal salt concentration for growth, CDM (pH 6.5) was supplemented with 0, 15, 33 or 45 g L^{-1} marine salts (Meersalz, Wiegandt GmbH, Krefeld, Germany), and culture plates were incubated at 25 ^oC in the dark. These concentrations included those of the natural habitats

(Table 3), which vary from ~ 1.0 % (Baltic Sea, Rügen) to >3.7 % (central Mediterranean Sea). We used artificial seawater or marine salts since Rohrmann *et al.* (1992) had reported no differences in growth and enzyme activities of several marine fungi including *D. salina* comparing culture on natural and artificial seawater.

Temperature. To determine the temperature for optimum growth, CDM (pH 6.5) was supplemented with 33 g L^{-1} marine salts, the most common salt concentration in seawater, and the cultures were incubated in the dark at 5, 15, 18, 22, 25, 30, 34 or 37 °C. These temperatures include those of their natural habitats (Table 3), which can range from 2 - 5 ° C (Baltic Sea in the winter) to 29 °C (Gulf of Mexico in the summer).

pH-value. To check for the pH requirement, aliqout volumes of CDM with 33 g L^{-1} marine salts were adjusted to pH 5.0, 6.5, 7.0 or 8.0 with 1 M HCl or 1 M NaOH. All cultures were incubated at 25 °C in the dark.

Varied combinations of salinity and temperature. To determine the combined influence of salinity and temperature, the *Dendryphiella* strains were grown on CDM (pH 6.5) supplemented with 0, 15, 33 or 45 g L^{-1} marine salts and incubated at 18, 25, 30 or 34 °C in the dark.

D.2. Physiological responses to biotic factors

The physiological responses of marine *Dendryphiella* strains to biotic factors were studied by determining their ability to produce biologically active secondary metabolites. Organisms may produce these secondary

metabolites in response to their environments, e.g. to protect themselves from competitors, and thus, may play important role in their survival.

Production and extraction of secondary metabolites. The 16 strains of *Dendryphiella* (those listed in Table 3) were initially grown on slants of Potato Carrot Agar (PCA; Höller *et al.*, 2000) with 33 g L^{-1} marine salts for 10 days at 25 °C. Spore suspensions used as inocula from these cultures were prepared in volumes of 50 mL 0.01% Tween (80) Water (TW). Approximately 5 mL inoculum for each strain was pour-plated with the following five agar culture media with 33 g L^{-1} marine salts at pH 6.5: (a) CDM with 3 % (w v^{-1}) carbon source (glucose, CG; sucrose, CS; or mannitol, CM) + 0.3 % (w v^{-1}) $NaNO_3$ as nitrogen source; (b) CDM with 3% (w v^{-1}) sucrose as carbon source + 0.3 % (w v^{-1}) nitrogen sources ($NaNO_3$, CS; NH_4NO_3, CN; peptone, CP; or yeast extract, CY); (c) Malt Extract – Peptone – Yeast Extract Agar (MPY: per litter with 20 g malt extract, 2.5 g yeast extract, 2.5 g peptone from meat and 12 g agar; Schulz *et al.*, 1995); (d) Malt Extract Agar (MEA: per liter with 20 g malt extract, 0.1 g yeast extract, 13 g agar); (e) Potato Dextrose Agar (PDA; Merck, Darmstadt, Germany). Other *Dendryphiella* strains were grown only on MPY with 33 g L^{-1} marine salts (without marine salts for terrestrial strains); strains listed in Table 2. All cultures were incubated for 4 weeks at 25 °C. Additional petri dishes with CDM with sucrose + sodium nitrate were inoculated and also incubated at 18, 25, 30 °C to determine the influence of temperature on production of secondary metabolites. After incubation, the cultures were lyophilized, ground, extracted with buffered ethylacetate and evaporated *in-vacuo*. Dried

crude extracts were then dissolved in 1:1 methanol – acetone (1.5 ml per 3 plates) and stored at 15 ^{o}C until use for assay.

Assay for biological activities. In assaying the crude extracts for bioactivity, the microorganisms were cultured as follows: *Chlorella fusca* on CP-Medium (CP: per liter 10 g yeast extract, 10 g D-glucose, 15 g agar; Schulz *et al*., 1995), but at pH 6.2, for 4 days under white light at 22 ^{o}C; *Microbotryum violaceum*, *Saccharomyces cerevisiae* and *Cladosporium cucumerinum* on MPY Agar (pH 6.5) for 4 days (7 days for *C. cucumerinum*) at room temperature (≈ 22 ^{o}C); *Bacillus megaterium* and *Escherichia coli* on Nutrient Agar (NA = NB in Schulz *et al*., 1995, and with 7.8 g peptone from meat, 7.8 g peptone from casein, 5.6 g NaCl, 2.8 g yeast extract, 1.0 g D-glucose and 12 g agar per 1 L distilled water, pH 7.5) for 24hr at 37 ^{o}C and the marine bacterium *Vibrio alginolyticus* on NA (pH 7.0) with 3 % (w v^{-1}) NaCl for 24hr at room temperature. Inocula were suspended in sterile distilled water (with 3 % NaCl for *V. alginolyticus*) and their titers standardized photometrically at 620 nm – 0.7 and 0.1 for the test alga and fungi, respectively, and 0.05 and 0.10 for the gram-negative and gram-positive bacteria, respectively. Each inoculum of test microorganism was sprayed onto antibiotic paper disks (Schleicher & Schuell, Dassel, Germany, 9 mm in diameter) impregnated with 50 uL of crude extract on agar medium. Culture media and conditions of culture were optimal for the respective test organisms. The zone of inhibition was measured with a Vernier caliper (3 readings per disk). Inhibition zones of the control (solvents and extracted uninoculated culture media) were deducted from those of the culture extracts.

TLC Bioautography. The bioactive metabolites of these strains were detected by TLC bioautographic overlay assay of strains grown on MPY with marine salts that exhibited moderate to strong activity against the test fungus *M. violaceum*. A cell suspension of pre-grown *M. violaceum* was photometrically (620 nm) adjusted to 0.1, mixed with pre-cooled MPY Agar to a concentration of 10 ml per 100 ml medium and poured onto TLC plates contaning 20 μL crude extracts which has been previously run 2-dimensionally with 4 % and 8 % MeOH in dichloromethane. The TLC overlays were then incubated for 4 days at 22 ^{o}C, the clear zones determined and compared with TLC plates sprayed with reagents.

III. Results

A. The marine *Dendryphiella* species, *D. arenaria* and *D. salina*

Following surface-sterilization of living and decaying algal and plant materials collected from different geographical locations and climatic zones, a total of 86 strains of marine *Dendryphiella* species were isolated (see Appendix A for a list of the isolates). Additional eleven strains were obtained from the culture collections in Canada (UAMH), the Netherlands (CBS) and Japan (NBRC). Of these 97 strains, 57 were then grown as monospore cultures and identified as *D. arenaria* (22 strains) and *D. salina* (35 strains) by conidial morphology and later by analysis of their ITS1 and 2, *tef*1 and *rpb*2 gene sequences (Table 2). Most strains identified morphologically were in concordance with their identification by gene sequences, though six strains from the Meditteranean Sea had conidial morphology characteristic of *D. arenaria*, but were later identified by gene analysis as *D. salina*. Strains from the sub-tropical Gulf of Mexico were mostly identified as *D. arenaria*, while the temperate European Atlantic Coast (North Sea, Germany and English Channel, United Kingdom) harbored mainly *D. salina* strains.

Analysis of the morphocultural characters of *D. arenaria* and *D. salina* revealed differences between the two species. Strains of *Dendryphiella* differed in their conidial morphology, e.g. mean spore length, with *D. arenaria* producing shorter conidia (< 20 μm) than *D. salina* (≥ 20 μm) (Fig. 4.A; see also Appendix C). There was also significant variations in the mean spore lengths between strains of one species. The spore lengths of both species were in agreement with the description provided by Pugh & Nicot (1964) and

Kohlmeyer & Volkmann-Kohlmeyer (1991). No major differences, however, were observed in their mean spore width (5 – 8 μm), rendering this character not suitable in discriminating the two species. The number of septa also did not differ greatly between the two species (mostly 1 – 4); though *D. salina* strains produced also conidia with more than 4 septa.

All 55 strains of *Dendryphiella* grew on MEA and PDA culture media supplemented with 33 g L^{-1} marine salts at 25 °C in the dark. Their mean colony extension rates did not vary much between the two culture media, but differed significantly between the two species and between strains of one species ($p < 0.001$, $n = 9$). Strains of *D. arenaria* exhibited faster mean colony extension rates than *D. salina*. The former had mcer ≥ 1.2 cm day^{-1} while the latter had mcer < 1.2 cm day^{-1} (Fig. 4.B, see also Appendic C).

However, identification by these morphocultural characters was not always in agreement with their identification by other characters. Six strains from the Mediterranean Sea, which were confirmed as *D. salina* by analysis of the gene sequences, exhibited mean colony extension rates similar to other strains of *D. salina*, but had mean conidial lengths characteristic of *D. arenaria*.

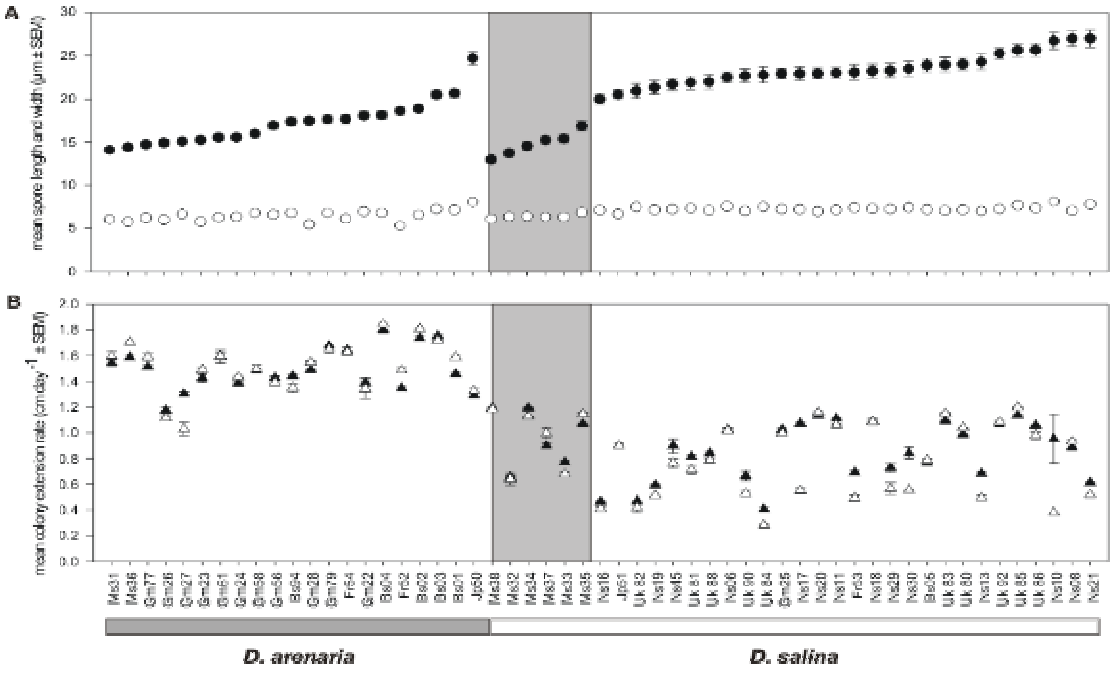


Figure 4. Conidial size (A) and growth on complex culture media (B) of *D. arenaria* and *D. salina*. Mean conidial length (●) and width (○) were measured from 100 spores per strain. The mean colony extension rates on MEAS (▼)and PDAS (▽) were computed from colony diameters measured after 3 and 5 days of incubation at 25 °C.

B. Genetic analysis of *Dendryphiella* and *Scolecobasidium* species

B.1. RAPD profiles of Dendryphiella and Scolecobasidium species

Amplification of the genomic DNA of marine *Dendryphiella* with the primer M13 revealed 13 RAPD band patterns which differed between and within the strains of the two marine species (Fig. 5). No RAPD band was specific for either species nor for their geographic origin, though the band patterns observed for the North Sea (06 – 21) and United Kingdom (80 – 92) isolates appeared similar. The band patterns of the individual *D. salina* strains from the Mediterranean Sea (32 – 35, 37 – 38) were also the same but differed from the other *D. salina* strains and from the two *D. arenaria*

strains (31, 36) of the same location. Strains of *D. arenaria* from the Gulf of Mexico (22 – 24, 26 – 28, 54 – 79) exhibited likewise similar RAPD profiles, irrespective of locality. Three different RAPD patterns, however, were observed among the five *D. arenaria* (01 – 04, 94) isolated from the Baltic Sea. Also, the RAPD profiles exhibited by the marine species differed clearly from those of the terrestrial *Dendryphiella* strains and from the representative strains of the genus *Scolecobasidium*. The RAPD profiles served only in identifying strains for subsequent gene sequencing.

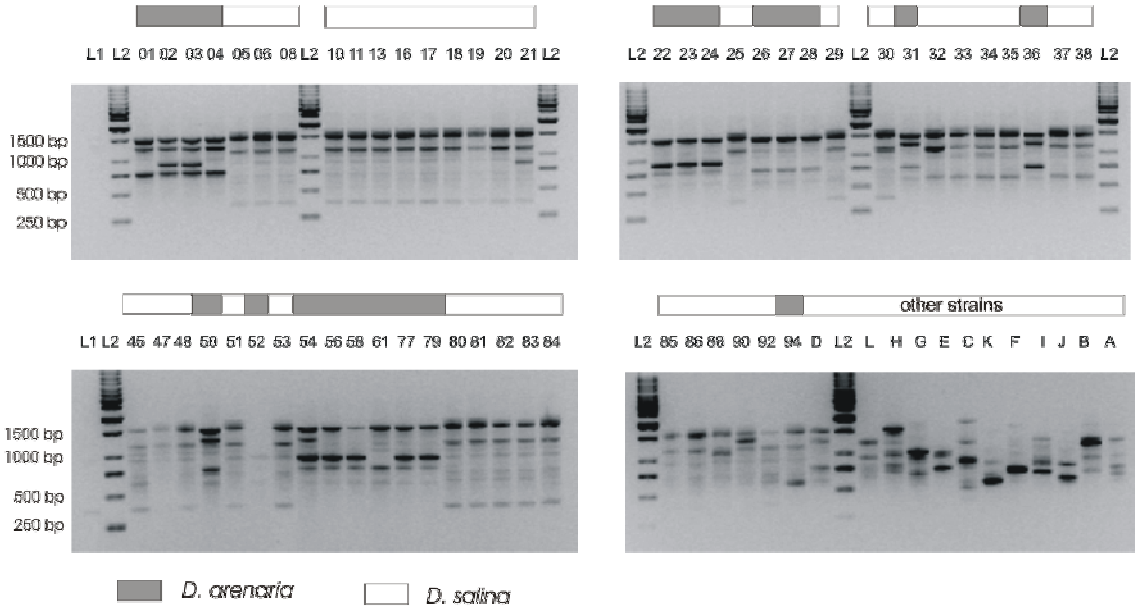


Figure 5. RAPD profiles of marine *Dendryphiella* species (01 – 94) and related terrestrial *Dendryphiella* (D, L) and *Scolecobasidium* (A – C, E – K) species. L1 represents the negative control while L2 represents the gene marker. See Table 2 for the geographical origin of the strains.

B.2. rpb2 sequence analysis of Dendryphiella and Scolecobasidium spp.

The nuclear gene *rpb*2, which encodes the second largest subunit of RNA polymerase II and has been used in ascomycete phylogeny (Liu *et al*., 1999), was sequenced for nine marine and two terrestrial strains of *Dendryphiella* and 10 representative strains of seven *Scolecobasidium* species. Analysis of this gene sequence revealed closely related but distinct clusters for *D. arenaria* and *D. salina*, irrespective of their geographic origin, with *Pleospora tarda* as the next taxonomic relative (Fig. 6). The two terrestrial strains, *D. vinosa* NBRC 32669 and *Dendryphiella* sp. NBRC 100153, did not occupy the same cluster with the marine species, but were taxonomically closer to the marine species than to the genus *Scolecobasidium*. The *Dendryphiella* species all belong to the Family *Pleosporaceae* while the 10 sequenced strains of *Scolecobasidium* formed a distinct genetically isolated phylogenetic group outside of the class *Laculoascomycetes* and were clearly distant from *D. arenaria* and *D. salina*.

Figure 6. Phylogenetic tree obtained from analysis of the *rpb*2 sequences of *Dendryphiella* and *Scolecobasidium* species. For GeneBank accession numbers of the strains, see Table 2. Other fungal gene bank accession numbers were obtained from Sweetser *et al*. (1987), Kawagishi *et al.* (1993), Liu *et al*. (1999), Chaverri *et al*. (2003), Reeb *et al*. (2004), Liu & Hall (2004), Miller & Huhndorf (2004, 2005) and Staats *et al.* (2005).

B.3. ITS1 & 2 and tef1 gene sequence analyses of D. arenaria and D. salina

The internal transcribed spacer (ITS 1 and 2) regions of the rRNA gene cluster were sequenced for thirty-six strains of *D. arenaria* and *D. salina*. Analysis of the ITS 1 and 2 showed the existence of two distinct species which correspond to *D. arenaria* and *D. salina* as identified by conidial morphology (Fig. 7). There were no correlations with geographical origin. The two marine species of *Dendryphiella* formed a sister clade and were taxonomically closely related to the genus *Pleospora* (anamorph *Stemphylium*). The two terrestrial *Dendryphiella* species were again distant from the marine species. The taxonomic position of *Dendryphiella* sp. NBRC 100153 remained uncertain, while *D. vinosa* NBRC 32669 was found to be closely related to *Preussia* spp. (Fig. 7).

The large intron of the translation elongation factor 1-alpha (*tef*1) was also sequenced for fourteen strains of *D. arenaria* and *D. salina*. Sequence analysis of the *tef*1 gene revealed two species of *Dendryphiella* irrespective of geographical origin (Fig. 8), similar to the results obtained from the phylogenetic analysis of ITS 1 and 2 (Fig. 7). Both marine species of *Dendryphiella* again formed sister clades. Different species of *Pleospora* and its anamorphic state, *Stemphylium*, were also found to be genetically closely related to the marine *Dendryphiella* and also belong to the family *Pleosporaceae* (Ascomycetes).

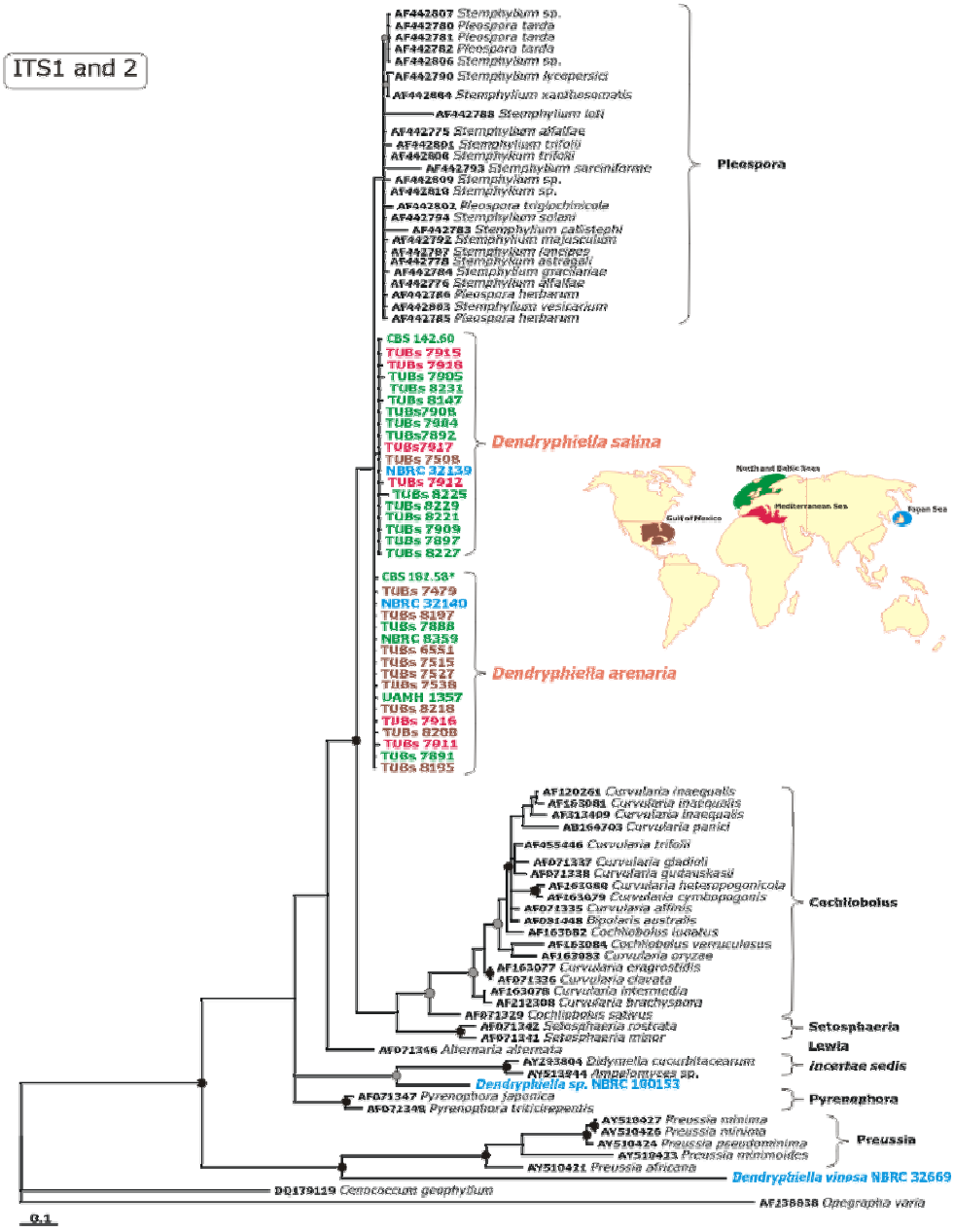


Figure 7. Phylogenetic trees obtained from analysis of ITS 1 and 2 gene sequences. For GeneBank accession numbers of *D. arenaria* and *D. salina* strains, see Table 2. For acccession numbers of *Pleospora* / *Stemphyllium* species, see Camara *et al*. (2002). Other species were obtained following nucleotide similarity search in GenBank. Geographic origins of the *Dendryphiella* species were marked brown for Gulf of Mexico, green for Baltic Sea, North Sea and European Atlantic Coast, red for Mediterranean Sea and blue for Japan.

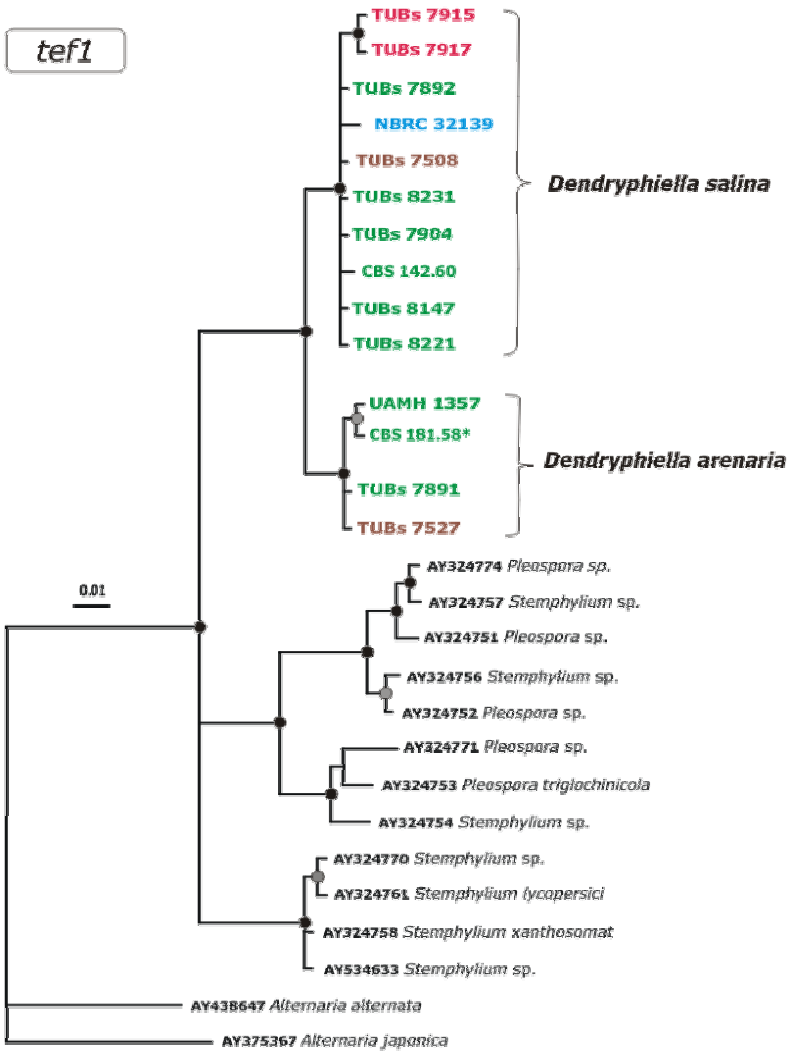


Figure 8. Phylogenetic trees obtained from analysis of *tef*1 gene sequences. For GeneBank accession numbers of *D. arenaria* and *D. salina* strains, see Table 2. Accession numbers of other fungal species, see Inderbitzin *et al.* (2005). Geographic origins of the *Dendryphiella* species were marked brown for Gulf of Mexico, green for Baltic Sea, North Sea and European Atlantic Coast, red for Mediterranean Sea and blue for Japan.

C. Phenetic analysis of marine *Dendryphiella* species

C.1. Carbon source assimilation by Dendryphiella

BIOLOG Phenotype MicroArray (PM) analysis of the 20 strains of *D. arenaria* and 33 strains of *D. salina* identified possible phenetic differences between the two marine species. Cluster analysis using complete linkage rule and Euclidean distance measure of the carbon source utilization profiles after 96 hours of incubation showed that the marine *Dendryphiella* strains formed 3 main clusters based on their phenotypes (Fig. 9), characterized by the simple matching coefficient values between 0.55 – 0.78 (Table 4). One of these clusters (2) had most of the strains of *D. salina*, mainly isolated from the European Atlantic Coast, i.e. from the North Sea, Germany, and from the English Channel, United Kingdom. Another cluster (1) contained only *D. arenaria*. Interestingly, several strains from the Baltic Sea (coastal areas, Germany) and the Gulf of Mexico formed distinct sub-groups within this cluster. Cluster 3 was subdivided into two sub-groups (3A, 3B) of which sub-cluster 3B contained only *D. salina* from the Mediterranean Sea and sub-cluster 3A had *D. arenaria* strains from different geographic origins. The conspicuous difference between the clusters 1, 2 and 3 was that strains of *D. arenaria* in cluster 1 ($n = 15$) grew faster on most carbon sources than strains of *D. arenaria* ($n = 5$) and *D. salina* ($n = 5$) present in cluster 3 (ANOVA, Holm-Sidak method, $p < 0.001$). Also, growth of *D. salina* strains from cluster 2 ($n = 27$) was even slower than that of *D. arenaria* (3A) and *D. salina* (3B) in cluster 3 ($p < 0.001$).

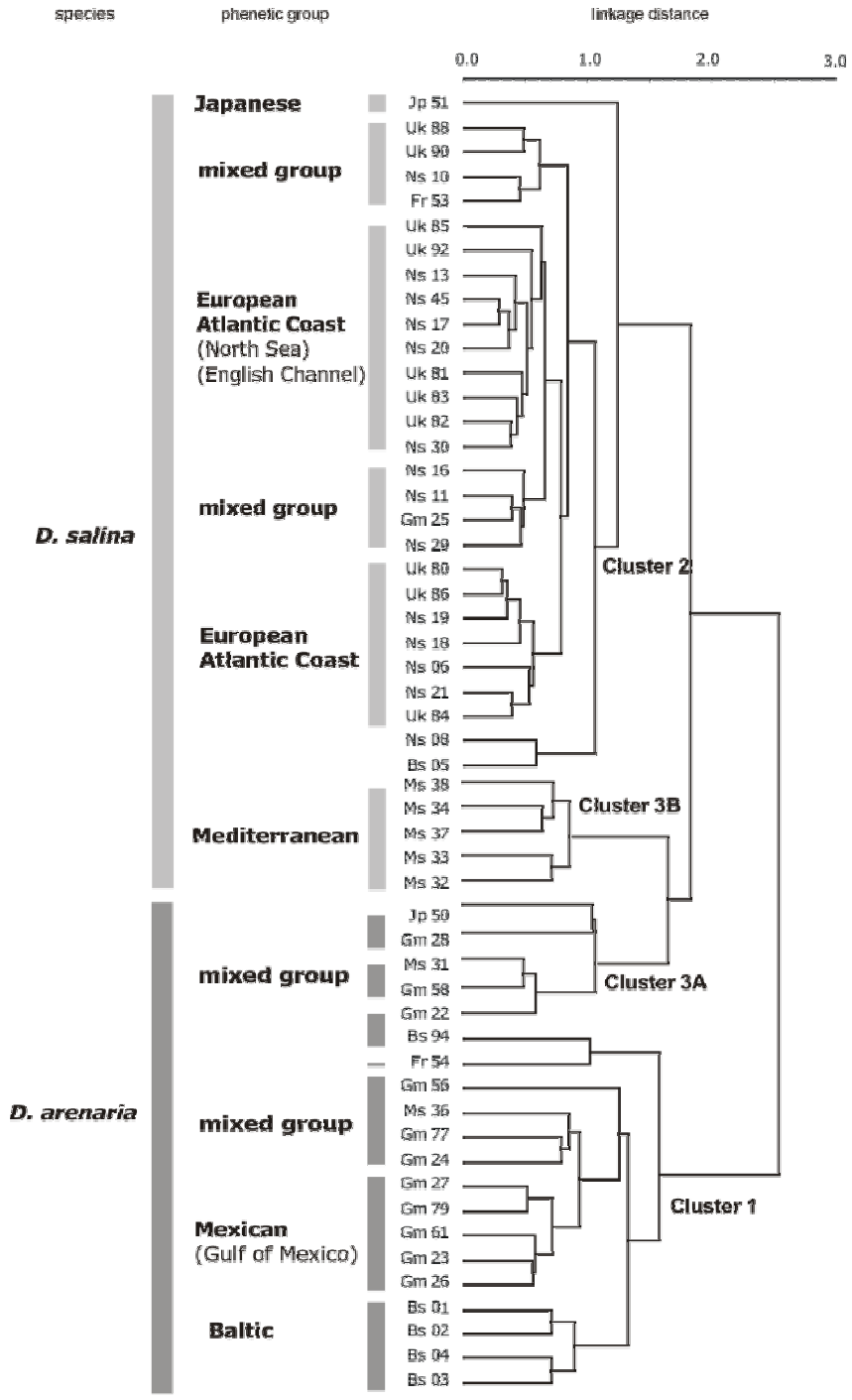


Figure 9. Correlations between carbon source utilization and geographical origin of marine *D. arenaria* and *D. salina* populations as revealed by the cluster analysis of the Phenotype MicroArray data after 96 hours of incubation. Clusters 1 and 2 represent a homogenous group of *D. arenaria* and *D. salina*. Cluster 3 includes both species. *D. arenaria* from the Baltic Sea (Germany) in cluster 1 and *D. salina* from the Mediterranean Sea (Malta, Greece) in cluster 3 formed distinct sub-groups.

Table 4. Similarity coefficient (simple matching coefficient) of marine *Dendryphiella* species based on BIOLOG and API ZYM profiles.

	Carbon Assimilation Profiles (BIOLOG Phenotype Microarrays)					**Constitutive Enzyme Profiles** (API ZYM)			
	Cluster 1	**Cluster 2**	**Cluster 3A**	**Cluster 3B**		**Cluster 1**	**Cluster 2**	**Cluster 3A**	**Cluster 3B**
Cluster 1 *D. arenaria* (n = 15)	1.00				**Cluster 1** *D. arenaria* (n = 15)	1.00			
Cluster 2 *D. salina* (n = 27)	0.55	1.00			**Cluster 2** *D. salina* (n = 27)	0.84	1.00		
Cluster 3A *D. arenaria* (n = 5)	0.73	0.69	1.00		**Cluster 3A** *D. arenaria* (n = 5)	0.84	0.74	1.00	
Cluster 3B *D. salina* (n = 5)	0.78	0.64	0.67	1.00	**Cluster 3B** *D. salina* (n = 4)	0.79	0.89	0.68	1.00

[a] See Table 2 and Fig. 9 for a list of the individual strains present in each cluster.

To determine the type of substrata *Dendryphiella* species utilized best, 4 similar strains of *D. arenaria* (Bs 01, Bs 02, Bs 03, Bs 04), all isolates from the Baltic Sea and belonging to a single phenetic group, was selected as reference strains. The optical density values indicating mycelial growth on the different carbon sources by these strains were likewise subjected to cluster analysis and revealed, according to the resulting growth, four groups of substrate utilization (Fig. 10). These groups were also typically observed for all other strains, though with some subtle differences in cluster affiliation for some carbon sources (Fig. 11). Substrates in group 1 that resulted in fastest germination and growth were primarily monosaccharides and disaccharides, the primary amino acids alanine and glutamate, the polyol myo-inositol and the organic acid fumarate. Interestingly, succinamide and turanose (α-1,3-

glucosyl-fructoside), which are usually poorly assimilated by other ascomycete fungi that have been studied (Tanzer *et al*., 2003; Druzhinina *et al*., 2006), also belong to this cluster. Group 2, which was characterized by carbon sources resulting in good growth, also contained several monosaccharides and glucosides, the amino acids asparagine, aspartate and serine, and the TCA-cycle intermediates succinate and succinic acid methylester. Group 3 had moderate to poorly metabolizable compounds which resulted in considerable delay in growth. Group 4 contained substrates which were not utilized. A conspicuous characteristic of the *D. arenaria* reference strains (Bs 01 - 04) was their poor growth on D-mannitol and D-sorbitol.

A detailed comparison of the carbon sources utilized by the strains of *D. arenaria* and *D. salina* revealed intriguing differences (Fig. 11, 12). Growth of both clusters of *D. salina* on the glycoside arbutin, the sugars L-rhamnose and D-ribose, and the polyol myo-inositol was proportionally slower than that of *D. arenaria* (Fig. 11). Further, *D. salina* was unable to use quinic acid and xylitol (Fig 12), on which both clusters of *D. arenaria* grew well. Finally, most strikingly, *D. salina* grew much better on i-erythritol, D-mannitol and D-sorbitol than *D. arenaria*. *D. arenaria* utilized N-acetyl-D-glucosamine fairly well, whereas *D. salina* did not. Both species, though, were unable to use D-glucosamine and L-fucose as substrates, the latter a monosaccharide component of brown algal cell walls. A number of our *Dendryphiella* strains have been isolated from the brown algae *Laminaria digitata* and *Fucus* spp. (Table 2).

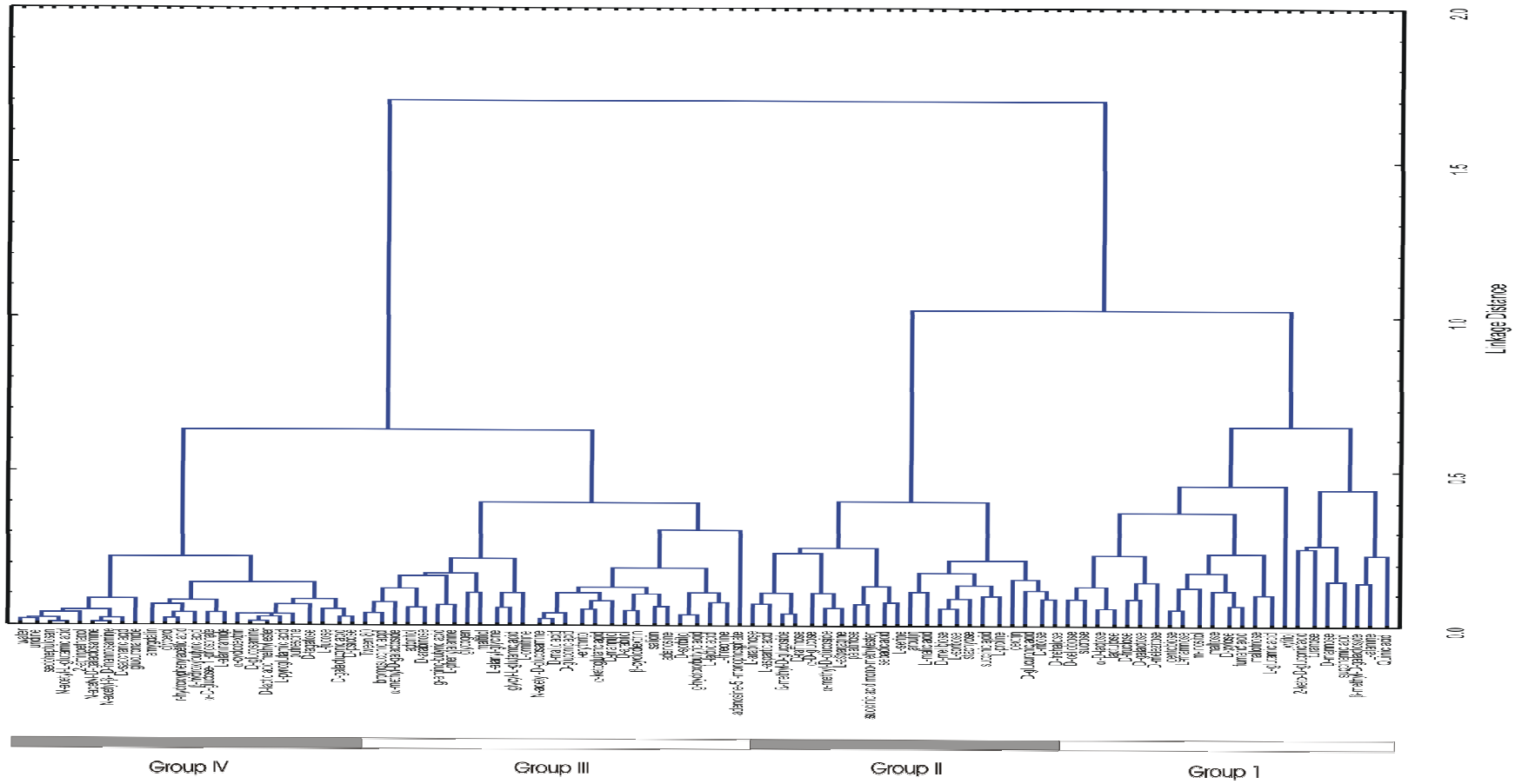


Figure 10. Cluster analysis of the substrates utilized by the reference strains *D. arenaria* Bs 01, Bs 02, Bs 03 and Bs 04. Cultivation on substrates in group 1, 2 and 3 resulted in fast, good and slow growth, respectively. Group 4 substrates were not utilized. Mycelial density (O. D.) was measured at 750 nm after 96 hours of culture.

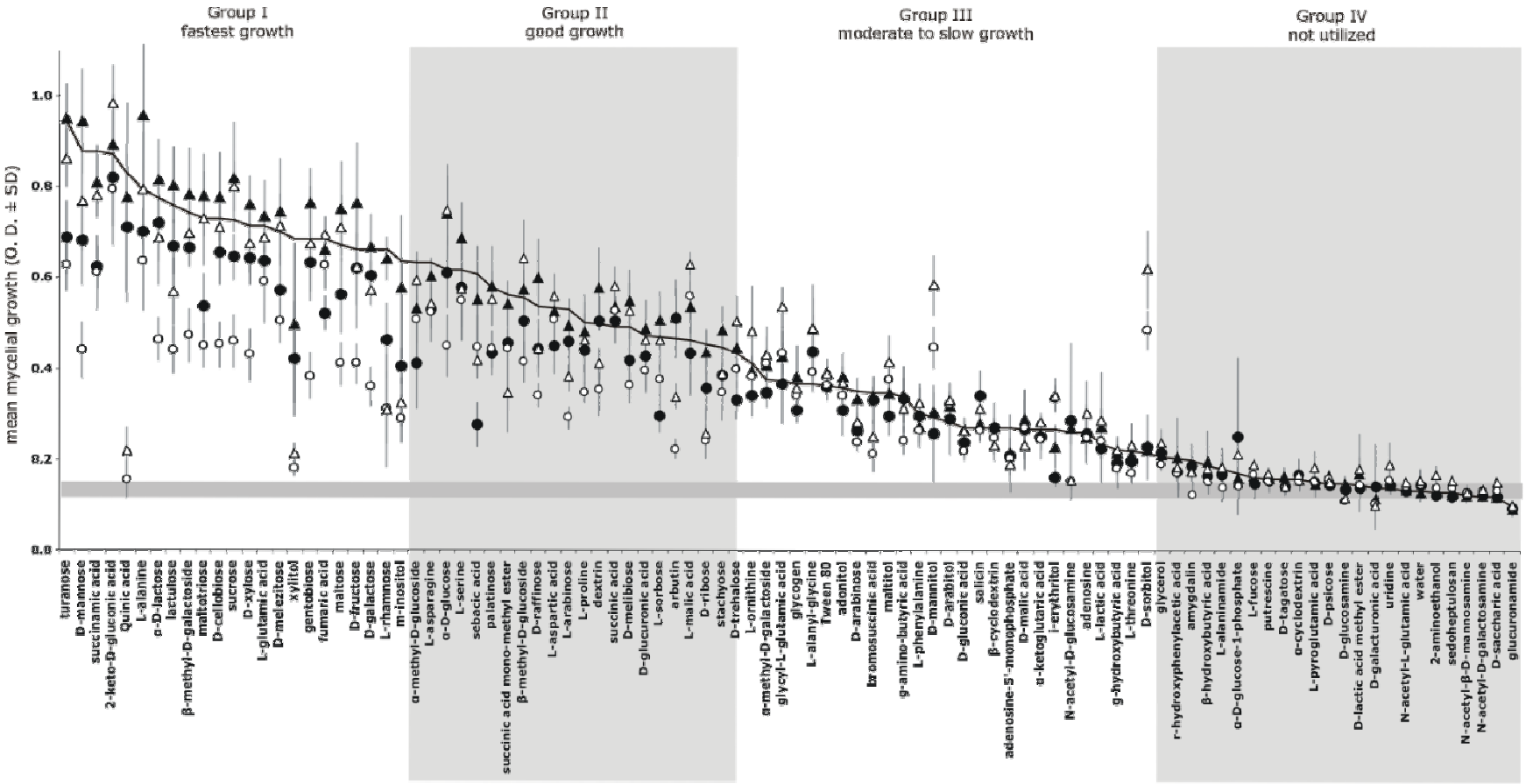


Figure 11. Carbon utilization profiles of *D. arenaria* and *D. salina* based on absorbance data (turbidity) at 750 nm following incubation of BIOLOG FF Microplates at 25 °C for 96 hours. Turbidity data measure mycelial growth, indicative of substrate utilization. *D. arenaria* Bs 01, Bs 02, Bs 03 and Bs 04 (—) were designated as reference strains in determining the group of substrates utilized by marine *Dendryphiella*. Filled circles and triangles correspond to *D. arenaria* clusters 1 (▼) and 3A (●) from Fig. 9, while open circles and triangles indicate *D. salina* clusters 2 (○) and 3B (▽). Statistical analysis (ANOVA) of the absorbance data revealed significant differences between the four clusters ($n = 4$; $p < 0.001$).

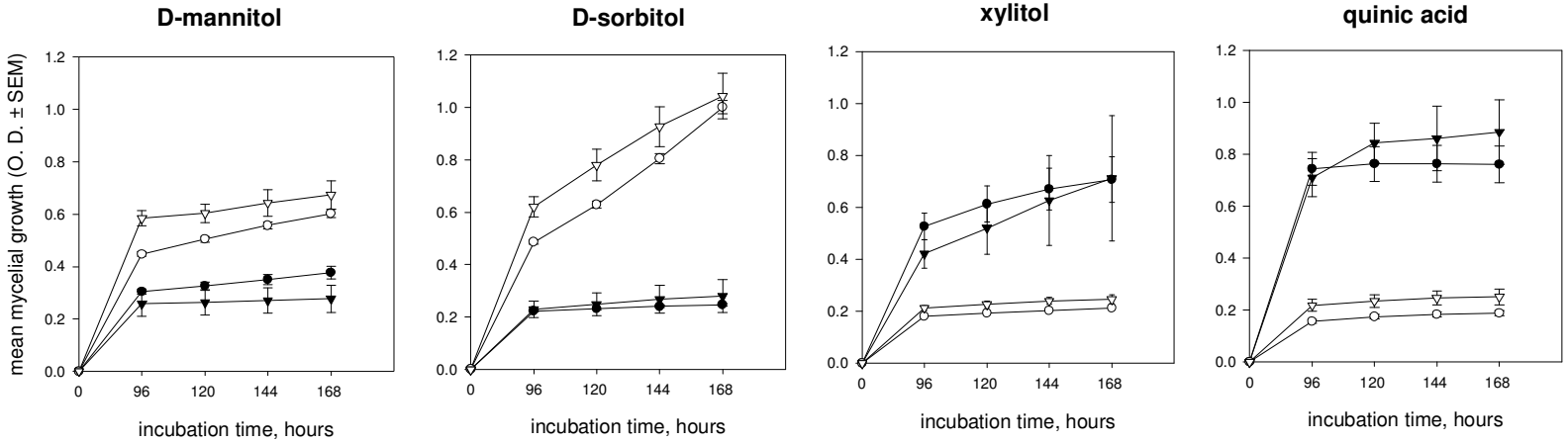


Figure 12. Utilization of selected carbon sources by *D. arenaria*, clusters 1 (●) and 3A (▼), and *D. salina*, clusters 2 (○) and 3B (▽), at 25 °C for 96 - 168 hours. The fastest growth was on substrates with an O. D. of > 0.65. Absorbance values between > 0.4 - 0.65 and > 0.2 - 0.4 represented good growth and moderate to poor growth, respectively. Mycelial growth with ≤ 0.2 O. D. indicated absence of substrate utilization.

Principal component analysis of utilization of the 14 most significant subtrates (measured as absorbance at 750 nm) grouped the strains into two clusters corresponding to *D. arenaria* and *D. salina* (Fig. 13). *D. salina* strains clustered together and were separated from those of the *D. arenaria*. As observed in cluster analysis (Fig. 9), *D. salina* strains from the Mediterranean Sea (32, 33, 34, 38) formed a group quite distant from the other *D. salina*, but definitely distinct from the *D. arenaria*. Four strains of *D. arenaria* (Ms 31 & 36, Gm 22 & 58) also had separate cluster, but still remained closer to the other strains of *D. arenaria*.

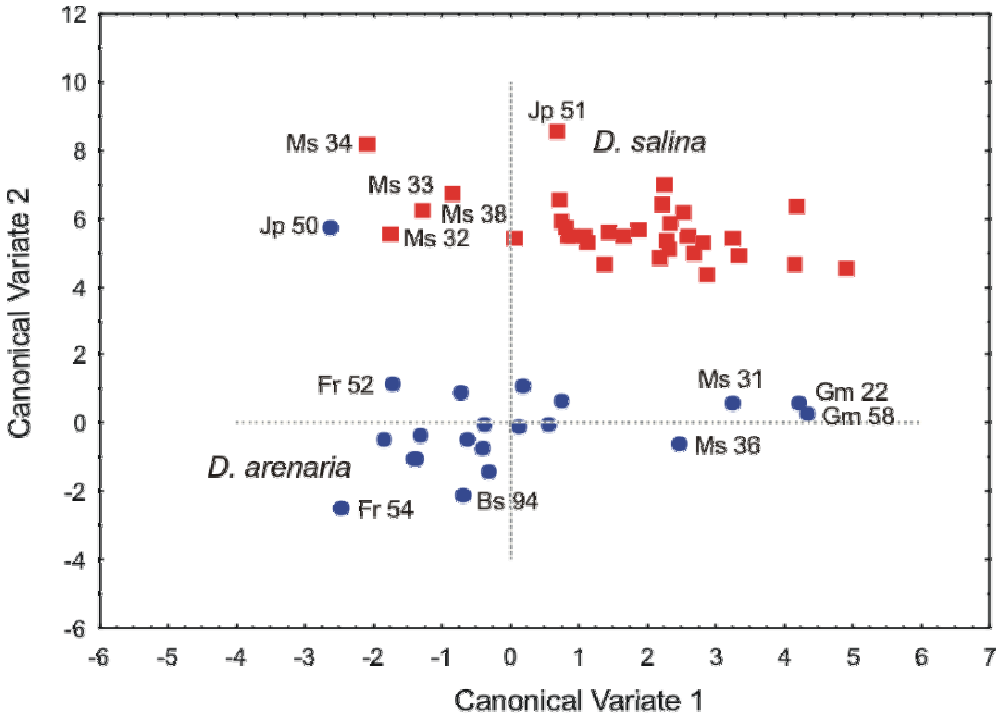


Figure 13. Principal component analysis of absorbance data at 750 nm of *D. arenaria* and *D. salina* strains after 96 hours of incubation.

The carbon source utilization profiles of the *ex*-type culture of *D. arenaria* (Fr 54 / NBRC 8359), which was obtained from the NBRC culture collection in Japan, were also tested. Contrary to our expections, this strain did not fully conform with the observed carbon utilization profiles of newly isolated strains of *D. arenaria*. Similar to *D. salina*, this strain was unable to grow on quinic acid but grew well on xylitol as the other *D. arenaria* strains did. To determine whether this was an artefact as a result of long-term subculturing or "domestication" of this strain (Fr 54 / NBRC 8359 had been maintained in culture for almost 50 years), we tested another strain of *D. arenaria* (Bs 94 / IBB TM 94) which had likewise been deposited in culture for a long time. This "domestication" effect may occur as a result of unintentional artificial selection due to the numerous *in vitro* reinoculation and/or revitalization of the fungal strains (Druzhinina *et al*., 2006). The profile of Bs 94 / IBB TM 94 fully confirmed the observed phenotype of Fr 54 / NBRC 8359, resulting in a separate branch in the cluster analysis within *D. arenaria* cluster 1 (Fig. 9). As also observed for *Hypocrea jecorina* QM 6a (Druzhinina *et al*., 2006), the *ex*-type culture of *D. arenaria* may no longer be representative of the species.

The strains of both *Dendryphiella* species also did not differ in their utilization of algal components and extracts and various sugars as carbon sources for growth in CDM with 3.3 % NaCl at pH 8. Mycelial growth was generally good with most tested C-sources with the exception of fucose and fucoidan (Table 5), both cell wall components of some Phaeophyceae. It was also poor on the extract of *Chondrus crispus* (Rhodophyceae).

Table 5. Utilization of algal components and extracts and various sugars by *Dendryphiella* species [a]

substrates	*D. arenaria*						*D. salina*									
	Bs [b]			Ms	Gm		Bs	Ms			Ns					Gm
	7888	7889	7891	7916	7541	7479	7892	7912	7914	7918	7893	7897	7898	7903	7909	7508
CDM w/o C sources	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
glucose, galactose fructose, mannose, xylose	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
sucrose	2	2	2	1	2	1	1	1	2	2	1	1	1	1	1	2
glucuronic acid	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0
fucose	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
fucoidan	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
alginic acid (1%, 3 %)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
laminarin	1	2	2	2	1	2	2	2	2	1	2	2	2	2	2	2
soluble starch	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
algal extract (*Laminaria digitata*)	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
algal extract (*Chondrus crispus*)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

[a] growth in microtiter wells
- 0 no mycelial growth
- 1 mycelial growth observed, wells partly covered
- 2 mycelial growth observed, wells completely covered

[b] origin of strains
Baltic Sea (Bs), Mediterranean Sea (Ms), North Sea (Ns), Gulf of Mexico (Gm)

C.2. Extracellular enzyme profiles of D. arenaria and D. salina

Analysis of the extracellular enzymes of 54 strains revealed similar enzyme profiles irrespective of species and geographical origin (Table 6 & 7). Strains of both *D. arenaria* and *D. salina* produced positive results for the enzymes amylase, lipase and urease and exhibited blackening of culture medium and clearing of opaque medium indicative of β-glucosidase and xylanase production, respectively (Table 6). However, other cellulolytic enzymes (cellulase, endoglucanase) were below the detection limit for all strains. Some activities, however, were confined only to some strains, e.g. laccase was only found in six strains of *D. arenaria*, four of which were from the Gulf of Mexico (Gm 23, Gm 26, Gm 56, Gm 58) and one each from the Mediterranean Sea (Ms 31) and Sea of Japan (Jp 50). Only two *D. arenaria* strains (Gm 22, Gm 56) reacted positively for peroxidase as detected by the syringaldazine agar well test. Polyphenol oxidase was produced by most strains (Table 6).

The *Dendryphiella* isolates utilised 10 or 11 of the substrates present in the API ZYM cupules (Table 7). Both *D. arenaria* and *D. salina* failed to produce the enzymes lipase (C14), valine- and cystine-arylamidases, the proteases trypsin and α-chymotrypsin, and the carbohydrases β-glucuronidase, α-mannosidase and α-fucosidase. There were no significant inter-cluster differences in the enzymes produced, though the amounts of substrates utilised varied. The mean intra- and inter-cluster similarity coefficient was between 0.68 – 0.89 (Table 4). The enzyme profiles were neither influenced by the use of distilled water with 3.3 % marine salts as the

inoculating suspension instead of distilled water per manufacturer's instructions, by incubating the API ZYM strips at 25 ^{o} C, nor by pre-growing the strains in complex culture media, e.g. Malt Extract Agar with 3.3 % marine salts (data unpublished).

Table 6. Extracellular enzyme production of marine *Dendryphiella* species on solid culture media.

	number of *Dendryphiella* strains with positive enzyme activity									
	D. arenaria				***D. salina***					
	Bs (4) [a]	Jp (1)	Gm (11)	Ms (2)	Bs (1)	Fr (1)	Gm (1)	Ms (6)	Ns (14)	Uk (10)
1. cellulolytic enzymes										
a. cellulase	0	0	0	0	0	0	0	0	0	0
b. endoglucanase	0	0	0	0	0	0	0	0	0	0
c. β-glucosidase	4	1	11	2	1	1	1	6	14	10
2. hemicellulolytic enzymes										
a. xylanase	4	1	11	2	1	1	1	6	14	10
3. lignin-modifying enzymes										
a. laccase	0	1	4	1	0	0	0	0	0	0
b. peroxidase	0	0	2	0	0	0	0	0	0	0
d. polyphenol oxidase	3	1	11	2	1	1	0	6	11	10
4. other extracellular enzymes										
a. amylase	4	1	11	2	1	1	1	6	14	10
b. lipase	4	1	11	2	1	1	1	6	14	10
c. urease	4	1	11	2	1	1	1	6	14	10

[a] number in parentheses represents the total number of strains tested. See also Table 2 for the origin of strains.
[b] Strains Bs 94, Fr 54 and Jp 51 were not included in this assay.

Table 7. Semi-quantitative enzyme activities of marine *Dendryphiella* species based on API ZYM data.

enzymes assayed for	quantity of substrate hydrolyzed [a]											
	D. arenaria					***D. salina***						
	Bs (5) [b]	Jp (1)	Fr (1)	Gm (10)	Ms (2)	Bs (1)	Fr (1)	Jp (1)	Gm (1)	Ms (6)	Ns (14)	Uk (10)
1. control	0	0	0	0	0	0	0	0	0	0	0	0
2. alkaline phosphatase	1	1	1	1	1	1	1	2	1	1	1	1
3. esterase (C4)	2 - 3	1	2	1 - 2	2	2	2	2	2	1 - 3	1 - 3	1 - 2
4. esterase lipase (C8)	1	1	1	1	1	1	1	1	1	1	1	1
5. leucine arylamidase	1 - 2	1	1	0 - 1	1	2	1	1	1	1	1 -2	1
6. acid phosphatase	1 - 3	3	2	1 - 2	1	1	1	3	1	1	1	1
7. Napthol-AS-BI-phosphohydrolase	0	1	0	0 - 1	0	0	0	1	1	0	0	0
8. α-galactosidase	1, 3	1	1	1	1	3	3	3	3	1 - 3	3	3
9. β-galactosidase	0	0	0	0 - 1	0 -1	0	0	0	0	0	0	0 - 1
10. α-glucosidase	1 - 2	1	1	1 - 2	1	1	1	3	1	1	1 - 3	1 - 2
11. β-glucosidase	3	3	3	3	3	3	3	3	3	3	3	3
12. N-acetyl-β-glucosaminidase	3	3	3	3	3	3	3	3	3	3	3	3

[a] Amount of substrate hydrolysed correlates with enzyme production.

0 no substrate hydrolysed
1 < 20 nanomole substrates hydrolysed
2 20 – 40 nanomole substrates hydrolysed
3 > 40 nanomole substrates hydrolysed

[b] Origin of strains (see also Table 2).

Baltic Sea (Bs), France (Fr), Gulf of Mexico (Gm), Japan (Jp),
Mediterranean Sea (Ms), North Sea (Ns), United Kingdon (Uk)

Number in parentheses represents the total number of strains tested. Strain Gm 28 was not included in this assay.

[C] The following enzymes were not synthesized by any of the strains:

- lipase (C14)
- valine arylamidase, cystine arylamidase
- trypsin, α-chymotrypsin
- β-glucuronidase, α-mannosidase, α-fucosidase

C.3. Secondary metabolic profiles of marine Dendryphiella species

Detection of the secondary metabolic profiles of the culture extracts of 22 *Dendryphiella* strains with thin-layer chromatography (TLC) following visualization with the general spray reagent H_2SO_4 in EtOH showed that the TLC profiles of *D. arenaria* and *D. salina* had similar pattern regardless of the origin of the strains (Fig. 14). Triterpenes and phenolic compounds, detected with the Cer-reagent and $FeCl_3$ in HCl, respectively, were present only in the culture extracts of five *D. salina* strains: Ms 7912, Bs 7892, Ns 7908, Fr 8147 and Uk 8229 (Fig. 15).

Thirty three peaks with different retention times, each representing a metabolite, were detected with HPLC-DAD in the crude ethylacetate extracts of all the *Dendryphiella* isolates (retention time up to 22 minutes). In the culture extracts of individual strains, however, 11 – 18 metabolites were detected. The profiles of the marine strains clearly differed from the terrestrial strains, e.g. *D. vinosa* (Fig. 16). Some of the metabolites (A – D, G) were produced by almost all of the strains, here represented by one strain each for *D. arenaria* and *D. salina* (Fig. 16, see also Appendix E). Four metabolites (H – K) were found to be more common in *D. salina* (present in at least 7 strains) than in *D. arenaria* (present only in 2 strains). But no metabolites were characteristic of only one of the species. The mean similarity coefficient based on their secondary metabolic profiles within *D. arenaria* and *D. salina* species was 0.54 and 0.47, respectively, and between the two species, 0.43 (Table 8). Its cluster analysis revealed four distinct groups, with three clusters containing both species (Fig. 17).

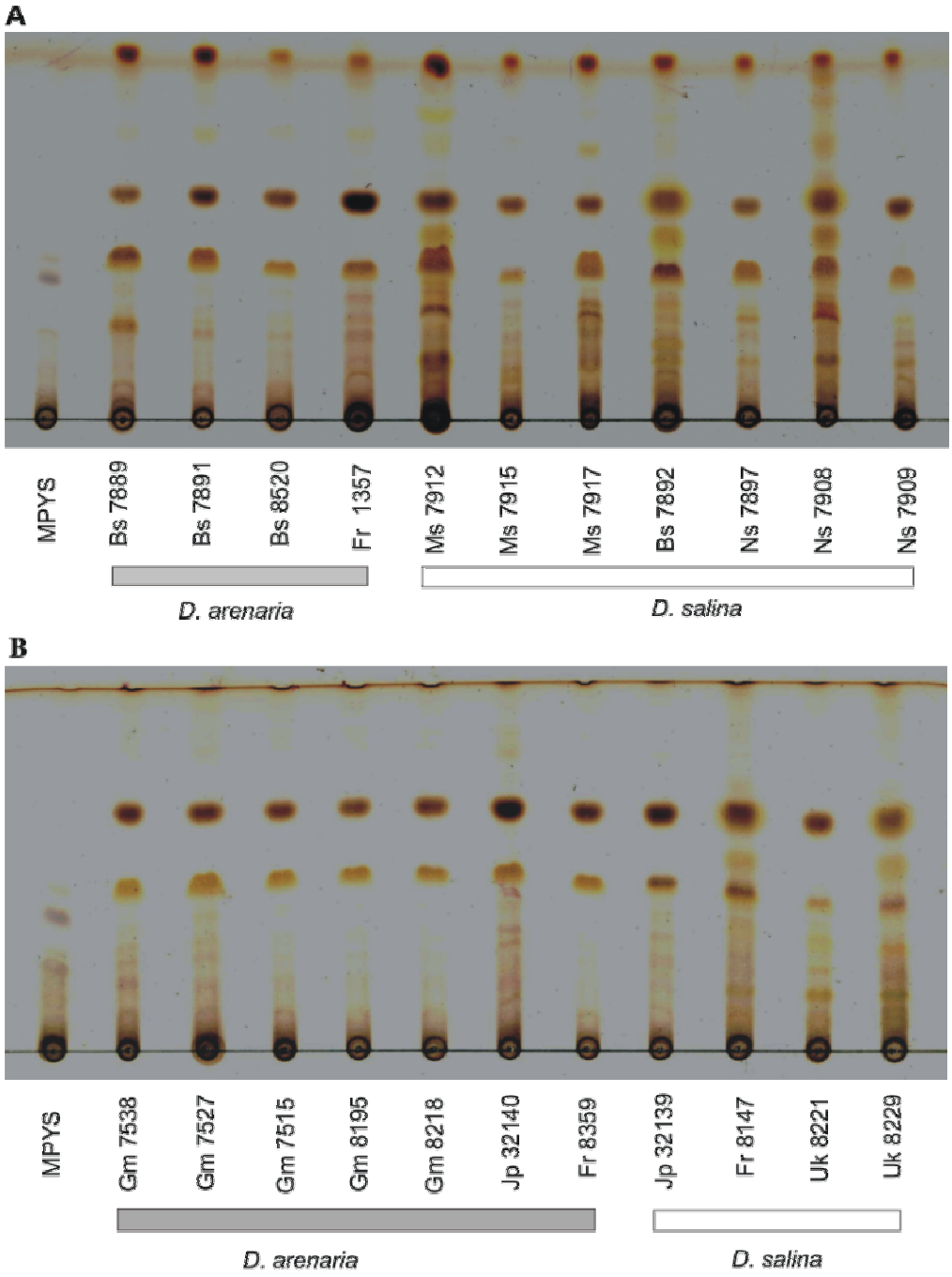


Figure 14. Thin layer chromatographic profiles of the crude culture extracts of marine *Dendryphiella* species. TLC plates (A, B) were visualized by spraying with 5 % H_2SO_4 in EtOH and subsequently heated for 10 min at 110 °C. Lightness, contrast and intensity of TLC plates were modified with Corel Photo-Paint Version 8. MPYS = Malt Extract – Peptone - Yeast Extract Agar supplemented with 33 g L^{-1} marine salts.

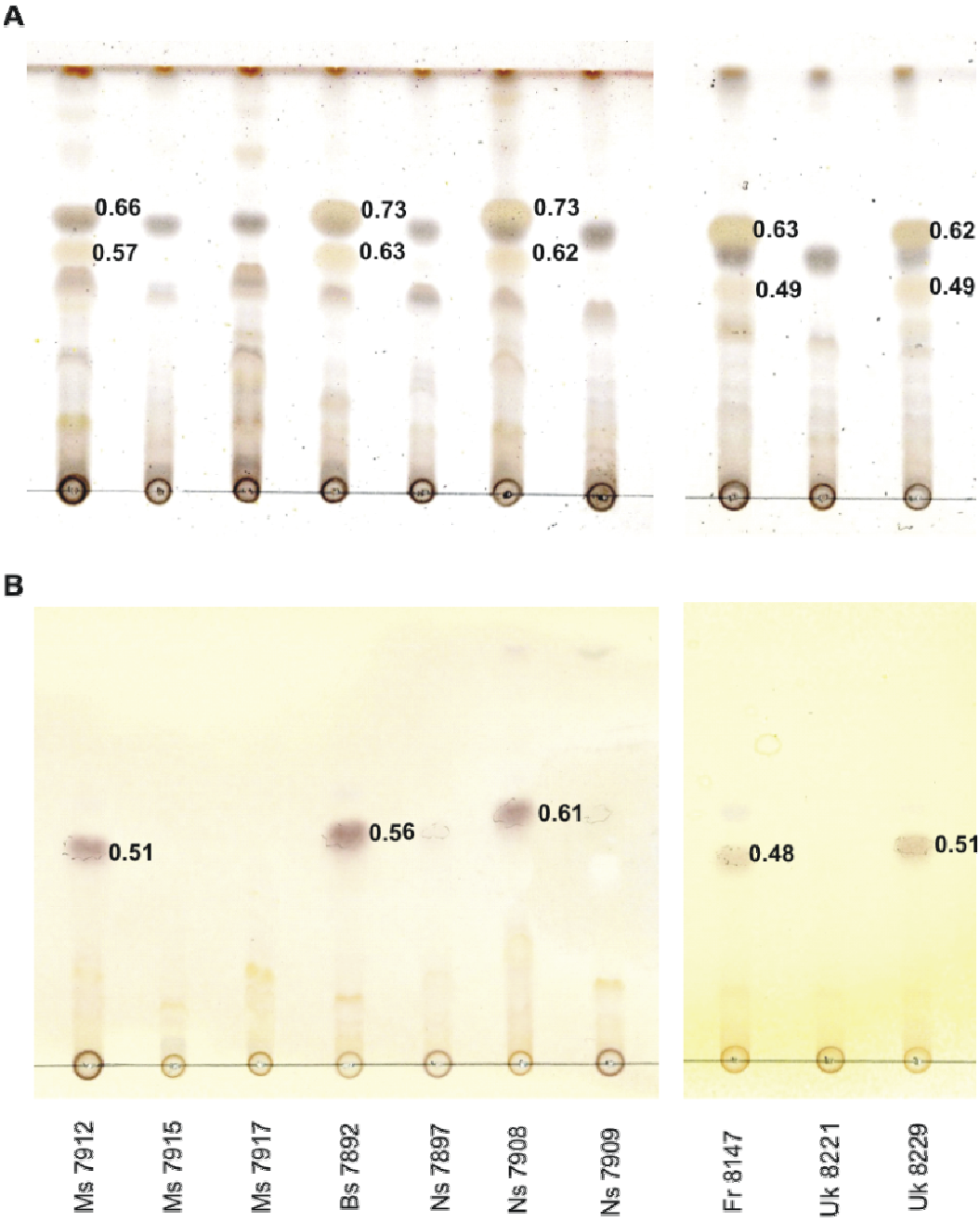
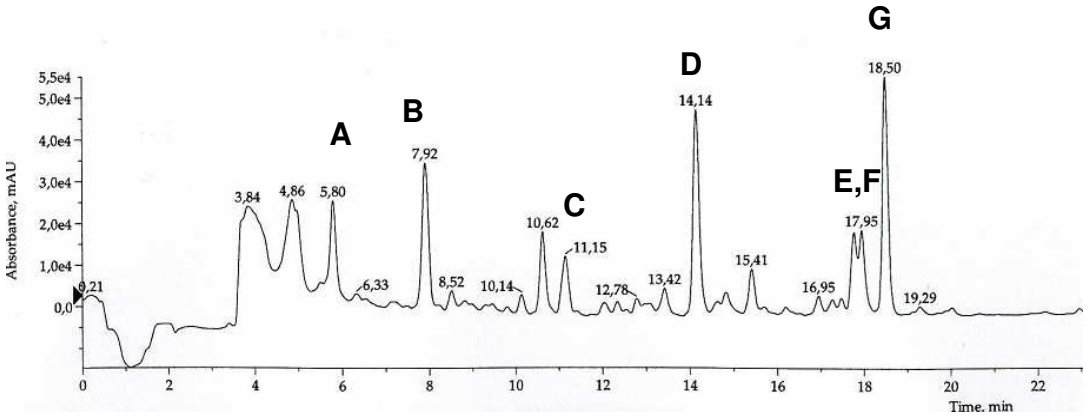
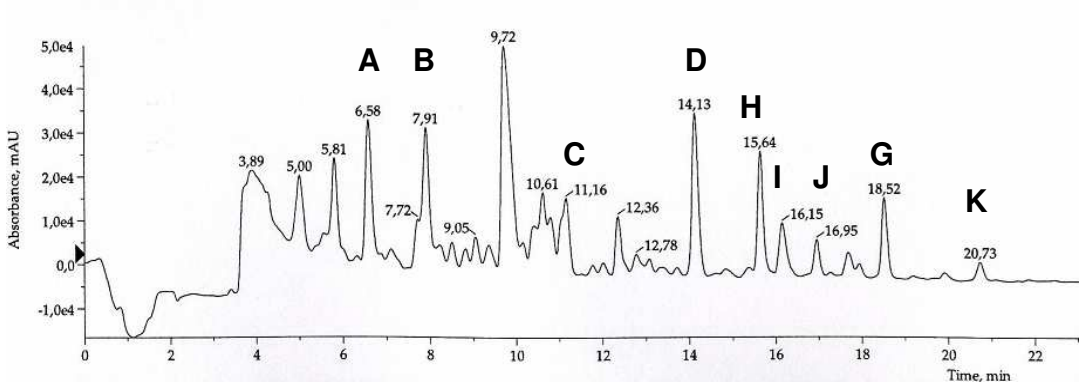


Figure 15. Thin layer chromatographic profiles of the crude extracts of *D. salina* strains. TLC plates were visualized by spraying with Cer-reagent (A) and $FeCl_3$ in HCl (B) and subsequently heated for 3 min at 120 °C and 60 °C, respectively. Rf values of the detected triterpenes (A) and phenolic compounds (B) were indicated in the TLC plates.

A. *D. arenaria* (TUBs 7889)



B. *D. salina* (TUBs 7897)



C. *D. vinosa* (NBRC 32669)

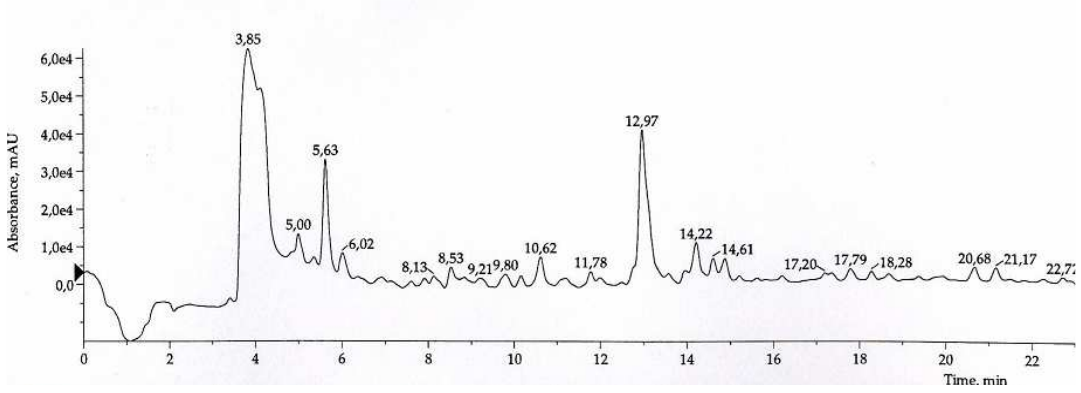


Figure 16. Secondary metabolic profiles of marine (A, B) and terrestrial (C) *Dendryphiella* species as detected by HPLC – DAD. Metabolites with their corresponding peaks were designated as A – K. Metabolite A, B, C, D and G were present in both *D. arenaria* and *D. salina*.

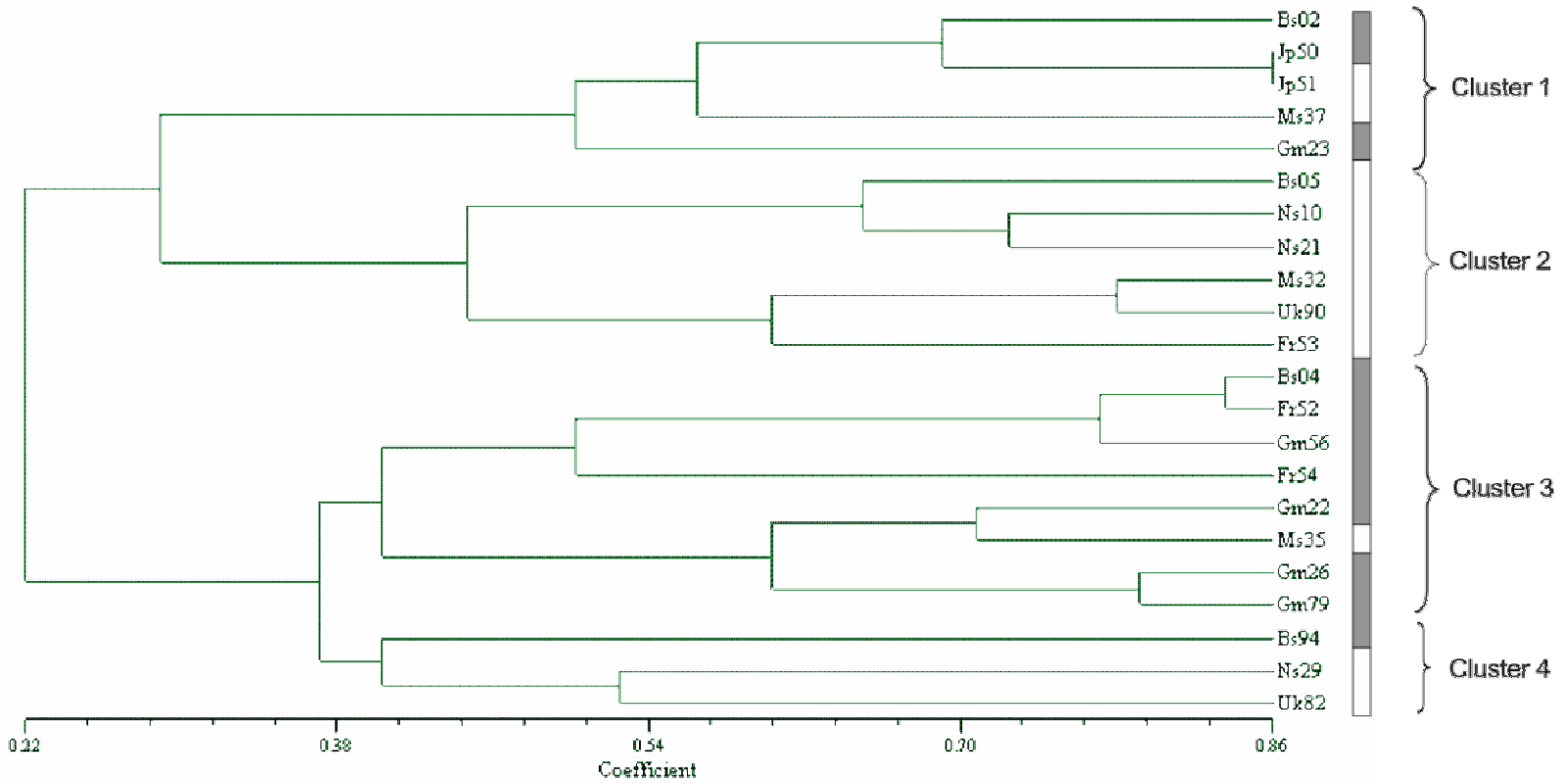


Figure 17. Phenogram of marine *Dendryphiella* species based on their secondary metabolic profiles (HPLC data). Similarity coefficient values (Jaccard's coefficient) among *D. arenaria* (gray-filled squares) and *D. salina* (white-filled squares) strains were between 0.22 – 0.86.

Table 8. Similarity coefficient values of secondary metabolic profiles (HPLC data) of marine *Dendryphiella* species from different geographical locations.

	D. arenaria											***D. salina***										
	Bs 02	Bs 04	Bs 94	Gm 22	Gm 23	Gm 26	Gm 56	Gm 79	Jp 50	Fr 52	Fr 54	Bs 05	Ns 10	Ns 21	Ns 29	Ms 32	Ms 35	Ms 37	Jp 51	Fr 53	Uk 82	Uk 90
Bs 02	1.00											**mean intraspecies similarity coefficient**										
Bs 04	0.56	1.00											*D. arenaria*				0.54 ± 0.13 (SD, n = 55)					
Bs 94	0.25	0.38	1.00										*D. salina*				0.47 ± 0.13 (SD, n = 55)					
Gm 22	0.45	0.53	0.37	1.00																		
Gm 23	0.58	0.50	0.35	0.55	1.00							**mean interspecies similarity coefficient**										
Gm 26	0.43	0.59	0.50	0.72	0.52	1.00							*D. arenaria* vs. *D. salina*				0.43 ± 0.12 (SD, n = 121)					
Gm 56	0.44	0.77	0.44	0.59	0.56	0.75	1.00															
Gm 79	0.45	0.53	0.38	0.65	0.70	0.79	0.67	1.00														
Jp 50	0.69	0.71	0.33	0.56	0.61	0.53	0.67	0.55	1.00													
Fr 52	0.56	0.83	0.38	0.53	0.50	0.59	0.77	0.53	0.71	1.00												
Fr 54	0.35	0.50	0.50	0.40	0.38	0.45	0.56	0.41	0.44	0.50	1.00											
Bs 05	0.39	0.38	0.29	0.30	0.42	0.29	0.35	0.32	0.33	0.38	0.50	1.00										
Ns 10	0.48	0.40	0.33	0.39	0.43	0.43	0.45	0.52	0.36	0.40	0.50	0.65	1.00									
Ns 21	0.33	0.32	0.25	0.26	0.36	0.25	0.30	0.33	0.29	0.32	0.50	0.67	0.72	1.00								
Ns 29	0.55	0.47	0.40	0.52	0.50	0.43	0.45	0.46	0.50	0.40	0.50	0.56	0.62	0.48	1.00							
Ms 32	0.35	0.33	0.33	0.33	0.50	0.38	0.38	0.52	0.36	0.33	0.58	0.56	0.62	0.55	0.42	1.00						
Ms 35	0.40	0.47	0.47	0.71	0.50	0.67	0.53	0.60	0.50	0.47	0.42	0.32	0.35	0.22	0.48	0.35	1.00					
Ms 37	0.56	0.69	0.29	0.44	0.50	0.42	0.53	0.45	0.60	0.57	0.50	0.47	0.40	0.39	0.56	0.47	0.39	1.00				
Jp 51	0.69	0.71	0.26	0.47	0.53	0.45	0.56	0.48	0.86	0.71	0.44	0.33	0.36	0.35	0.43	0.43	0.42	0.71	1.00			
Fr 53	0.38	0.30	0.24	0.25	0.55	0.24	0.29	0.38	0.33	0.30	0.47	0.44	0.45	0.53	0.39	0.60	0.26	0.37	0.40	1.00		
Uk 82	0.33	0.38	0.45	0.43	0.42	0.48	0.50	0.50	0.41	0.45	0.41	0.38	0.52	0.39	0.56	0.46	0.39	0.38	0.35	0.22	1.00	
Uk 90	0.45	0.37	0.30	0.30	0.55	0.29	0.35	0.43	0.40	0.37	0.56	0.73	0.68	0.61	0.52	0.78	0.32	0.44	0.40	0.67	0.38	1.00

[a] See Table 2 for the geographic origin of the strains.

D. Physiological responses of *Dendryphiella* to abiotic and biotic factors

D.1. Salinity, Temperature, pH

Salinity. All 16 *Dendryphiella* strains tested grew better with than without marine salts (15 – 45 g L^{-1}) as determined by their mean colony diameters and extension rates (Fig. 18.A). Most isolates grew optimally with 33 g L^{-1} marine salts (One-way ANOVA, Student-Newman-Keuls Method, $p < 0.001$), though some of them grew equally well with 15 – 45 g L^{-1} marine salts (Fig. 18.A). There were also differences among the isolates. The mean colony extension rates of *D. arenaria* in the various marine salt concentrations tested remained faster than *D. salina* – generally with mcer ≥ 1.1 cm day^{-1}, regardless of their geographical origin. Of these *D. arenaria* isolates, the three Baltic Sea (Bs) strains grew fastest at all marine salt concentrations (Fig. 18.A). The good growth of these Baltic Sea isolates even at the highest marine salt concentration tested (45 g L^{-1}) was somewhat surprising since the coastal waters of the Baltic Sea are less saline (approx. 10 g L^{-1}; Table 3). All strains sporulated at all salinities on CDM (data not shown).

Temperature. The 16 *Dendryphiella* strains grew on CDM (pH 6.5, 3.3 % marine salt) at temperatures between 5 – 34 ^{o}C, but not at 37 ^{o}C. The best growth occurred between 22 – 30 ^{o}C, with an optimum at 25 ^{o}C for most strains irrespective of geographical location and climatic zone (Fig. 18.B, $p < 0.001$). While at 15 ^{o}C growth rates among the strains of *D. arenaria* and *D. salina* slightly vary, at higher temperatures those of the former grew again faster than the latter ($p < 0.001$). This was most pronounced at 34 ^{o}C, where

D. arenaria strains reached mcer > 0.8 cm day^{-1}, regardless of their geographical origin.

Growth at 37 ^{o}C was completely inhibited and at 5 ^{o}C became visible only after 5 days of incubation (data not shown), resuming when the cultures were incubated at 25 ^{o}C; those that had initially attained minimal growth at 5 ^{o}C even reached the high growth rate typical of cultures started at 25 ^{o}C (data not shown). Spore formation also occurred in these cultures, as it did at all other temperatures.

pH. The isolates grew at all pH-values tested. Growth was good over a broad range from pH 6.5 to 8.0 (Fig. 18.C). There were no correlations between geographical origin and mcer at any of the pH - values. There were, however, differences between some strains of the individual species. The mean mcer of the *D. arenaria* strains was again greater than that of the *D. salina* strains ($p < 0.001$). The three *D. arenaria* isolates from the Baltic Sea had the greatest mean growth rates. All strains sporulated at all tested pH-values.

Salinity – temperature combination. All 16 *Dendryphiella* strains exhibited similar growth patterns in response to the combined influence of salinity and temperature, as illustrated in Figure 19 for one strain of *D. arenaria* and three strains of *D. salina*. As is the case for most marine fungi, at higher temperatures of culture the salinity optimum shifted to higher salt concentrations. This "*Phoma*-pattern of growth" (Richie, 1957) was very pronounced in cultures incubated at 34 ^{o}C. Such a response was observed regardless of the source of the strains. On the other hand, at a lower

temperature (18 °C), variations in growth were minimal, independent of the marine salt concentration. Growth of all strains was relatively poor in the absence of marine salts. Statistical analysis of the mean values of the mcer with Two-Way ANOVA (Holm-Sidak method) revealed significant interaction ($p < 0.001$) between the factors salinity and temperature.

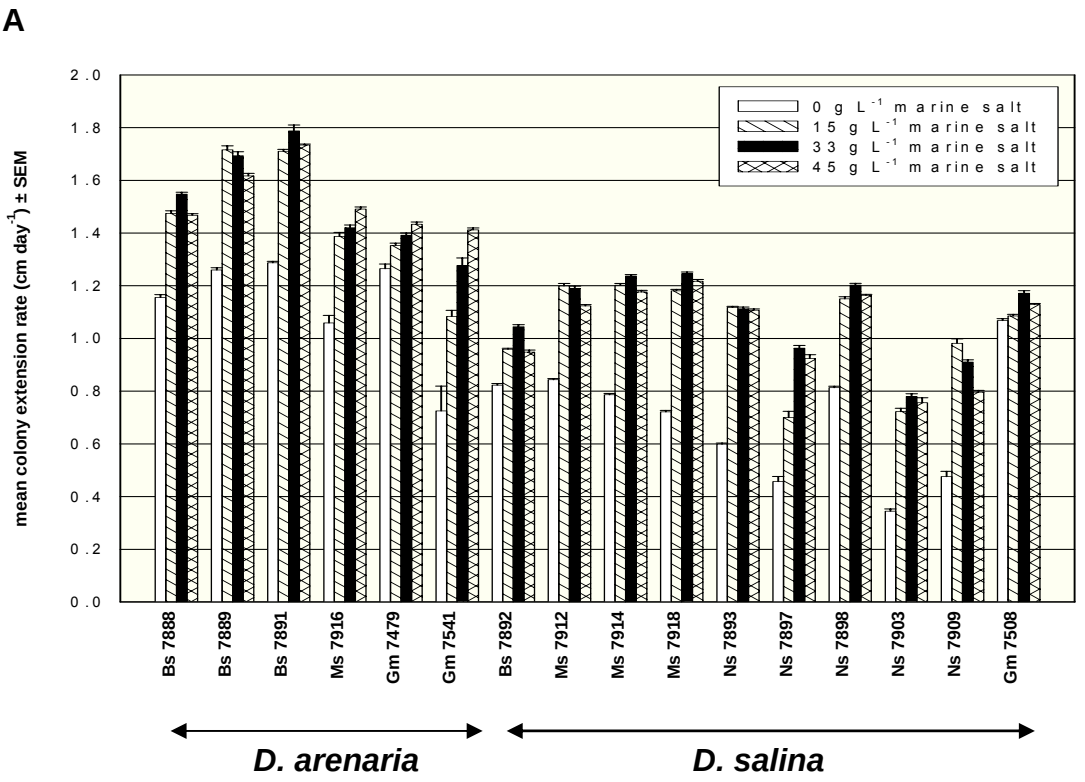
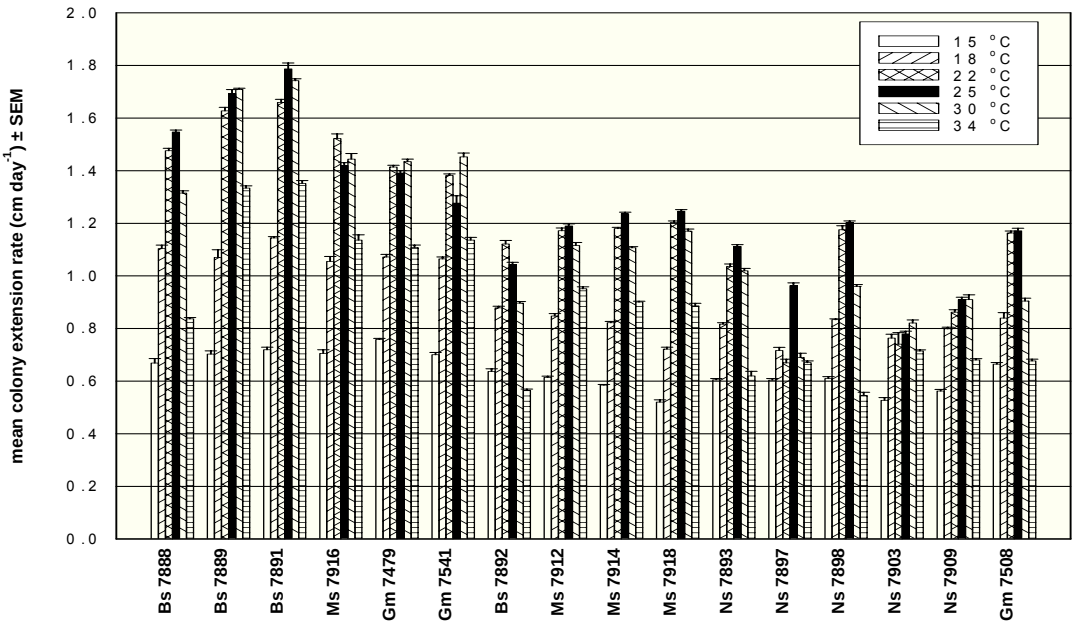


Figure 18. Growth of marine *Dendryphiella* species at different marine salt concentrations (A), incubation temperatures (B) and pH values (C) . Mean values within and between species are statistically significant ($p < 0.001$; $n = 9$). Geographical origins of the strains are represented as Bs (Baltic Sea), Ms (Mediterranean Sea), Ns (North Sea) and Gm (Gulf of Mexico).

B



C

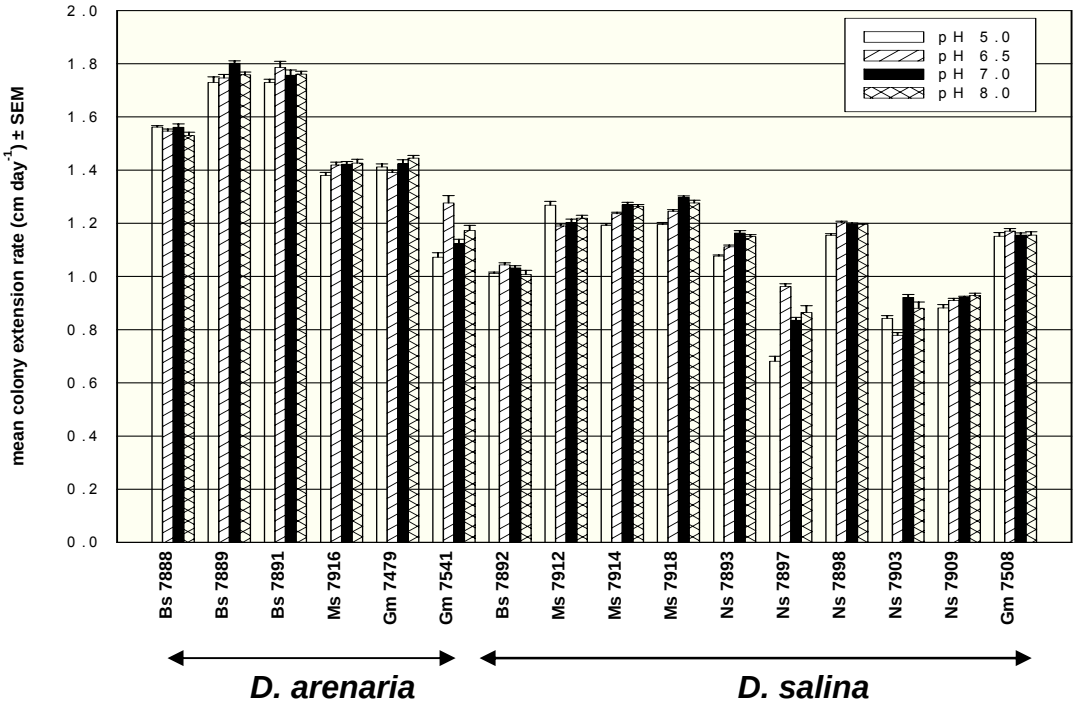


Figure 18. cont.

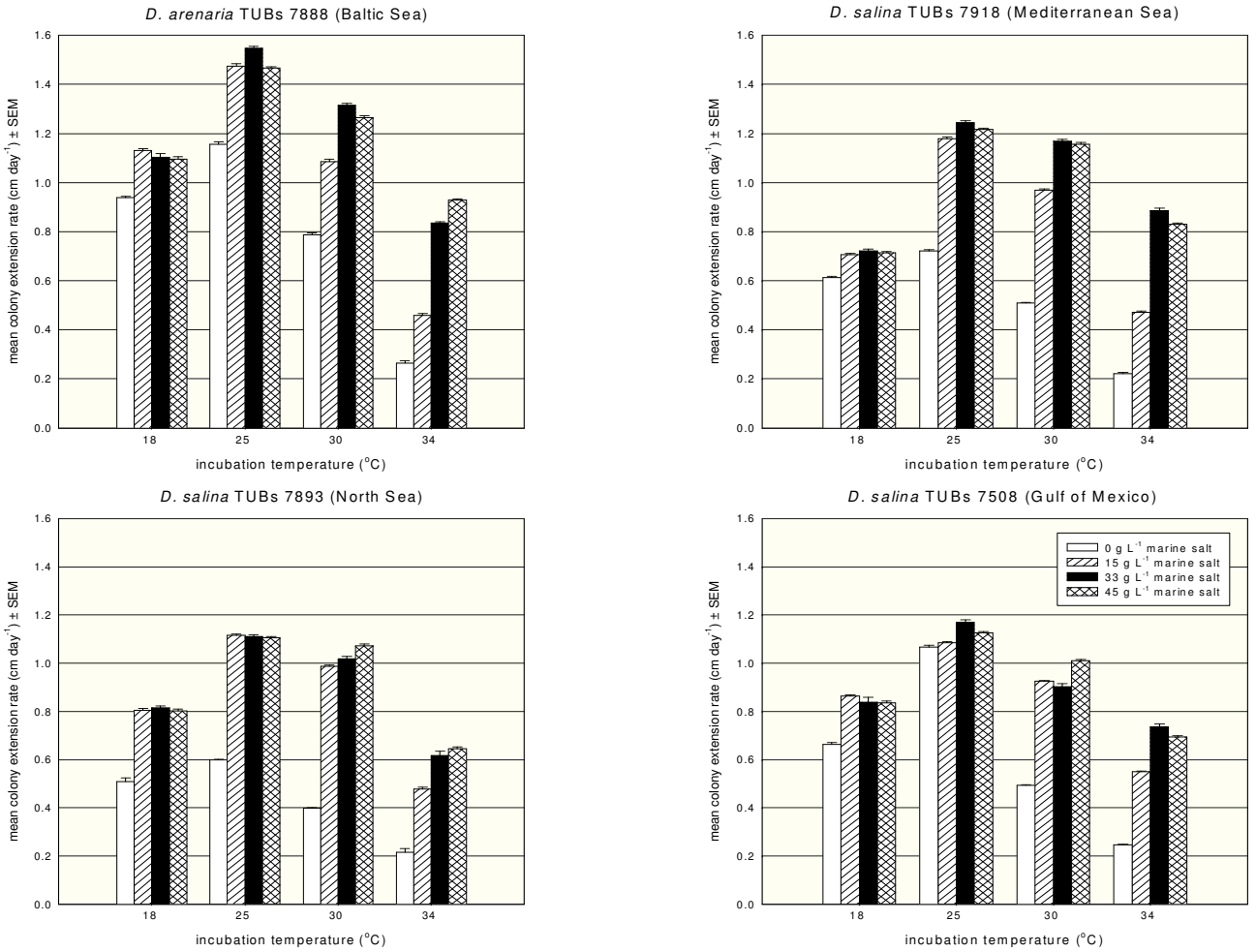


Figure 19. Combined influence of salinity and temperature on growth of *Dendryphiella* species. Mean values between conditions of culture per strain are statistically significant ($p < 0.001$; $n = 9$).

D.2. Antimicrobial Activities

Extracts from cultures grown at 25 °C. To test for antimicrobial metabolites, crude ethylacetate culture extracts of the 16 strains tested were assayed for inhibition of microbial test organisms. With the exception of *Escherichia coli,* each of the test organisms was inhibited by some isolate(s) of each species (Table 9 & 10, see also Appendix E for the biological activities of the individual strains). Inhibition was weak against *C. fusca*, *S. cerevisiae* and *V. alginolyticus*, and weak to moderate against *B. megaterium*. *M. violaceum* was inhibited by all the crude extracts, though the intensity of inhibition varied greatly (Table 10); *C. cucumerinum* was not inhibited. There were no correlations with species, geographical origin or climate zone, though there was great variation between the individual strains. The activities of the culture extracts from the different culture media did not differ greatly within each of the tested strains.

Extracts from cultures grown at 18 – 30 °C. To test for the possible influence of incubation temperature on the production of bioactive metabolites, the *Dendryphiella* strains were assayed for antimicrobial activity at different temperatures. Although the intensities of the inhibitions varied, incubation temperature had no significant generalized effect on the biological activity of the culture extracts (data not shown, see Appendix E). There were only strain specific.

Thin-layer chromatography and bioautography. To detect the bioactive secondary metabolites, selected *Dendryphiella* strains were assayed using TLC-agar overlay with the fungus *M. violaceum*. Clear zones

were only observed on bioautograms of culture extracts of the more active strains (Table 10), e.g. Bs 7892 and Ms 7917, and were located near the point of origin (Fig. 20.A), but not of those that had been weakly inhibitory, e.g. Bs 7891 and Ms 7912. 2-D TLC (Fig. 20.B) revealed that the culture extracts of Ms 7917 contained at least two bioactive metabolites. The metabolites, however, remained unidentified.

Table 9. Antialgal and antibacterial activity of crude ethylacetate culture extracts of *Dendryphiella* species grown at 25 °C on nine different media.[a]

species	Strain	diameter of zone of inhibition (in mm) [c]			
		C. fusca	*B. megaterium*	*E. coli*	*V. alginolyticus*
D. arenaria	Bs (3) [b]	0 – 12	0 – 18	0	0 – 22
	Gm (2)	0 – 16	8 – 26	0	0 – 10
	Ms (1)	0 – 12	11 – 24	0	0 – 20
D. salina	Bs (1)	0 – 14	9 – 13	0 – 2	0 – 14
	Ms (3)	0 – 14	7 – 24	0	0 – 18
	Ns (5)	0 – 17	6 – 28	0	0 – 20
	Gm (1)	0 – 12	7 – 25	0	0

[a] Metabolites tested were extracted from cultures grown on the following culture agar media:
- CDM with different C (CG, CS, CM) and N (CS, CN, CP, CY) sources
- complex media (MEA, MPY, PDA)

[b] geographical origin of strains, (x) – number in parenthesis indicates number of strains
Bs – Baltic Sea, Ns – North Sea, Gm – Gulf of Mexico, Ms – Mediterranean Sea

[c] diameter of zone of inhibition

0	no inhibition	20 – 30 mm.	moderate inhibition
< 20 mm.	weak inhibition	> 30 mm.	strong inhibition

Table 10. Antifungal activity of crude ethylacetate culture extracts of marine *Dendryphiella* species grown on MPY with 3.3 % marine salts at 25 °C (n = 3).

Den Nr. [2]	diameter of zone of inhibition in mm. [1]		
	S. cerevisiae	***M. violaceum***	***C. cucumerinum***
D. arenaria			
Bs 7889	0.1 – 0.4	0.5 – 1.0	0
Bs 7891	1.4 – 1.8	2.2 – 3.7	0
Bs 8520	3.5 – 7.1	3.4 – 4.8	0
Gm 7538	4.3 – 5.7	3.8 – 6.2	0
Gm 7527	3.8 – 5.6	2.9 – 5.8	0
Gm 7515	4.0 – 4.8	3.6 – 6.6	0
Gm 8195	5.2 – 6.0	3.4 – 4.1	0
Gm 8218	5.2 – 6.0	1.1 – 2.5	0
Jp 32140	1.7 – 4.3	2.6 – 2.9	0
Fr 1357	2.4 – 4.8	2.0 – 2.3	0
Fr 8359	1.1 – 2.2	3.2 – 4.0	0
D. salina			
Bs 7892	5.4 – 6.8	20.2 -21.0	0
Ms 7912	1.7 – 2.9	0.5 – 1.5	0
Ms 7915	2.2 – 2.6	20.2 – 21.7	0
Ms 7917	1.4 – 2.9	35.6 – 37.8	0
Ns 7897	2.4 – 5.0	0.6 – 1.0	0
Ns 7908	0.8 – 1.5	27.1 – 28.5	0
Ns 7909	5.0 – 5.2	30.4 – 33.4	0
Jp 32139	2.2 – 3.0	0.8 – 1.7	0
Fr 8147	0.9 – 2.0	2.2 – 4.7	0
Uk 8221	1.2 – 2.4	19.0 – 20.3	0
Uk 8229	2.1 – 3.0	20.4 – 22.7	0

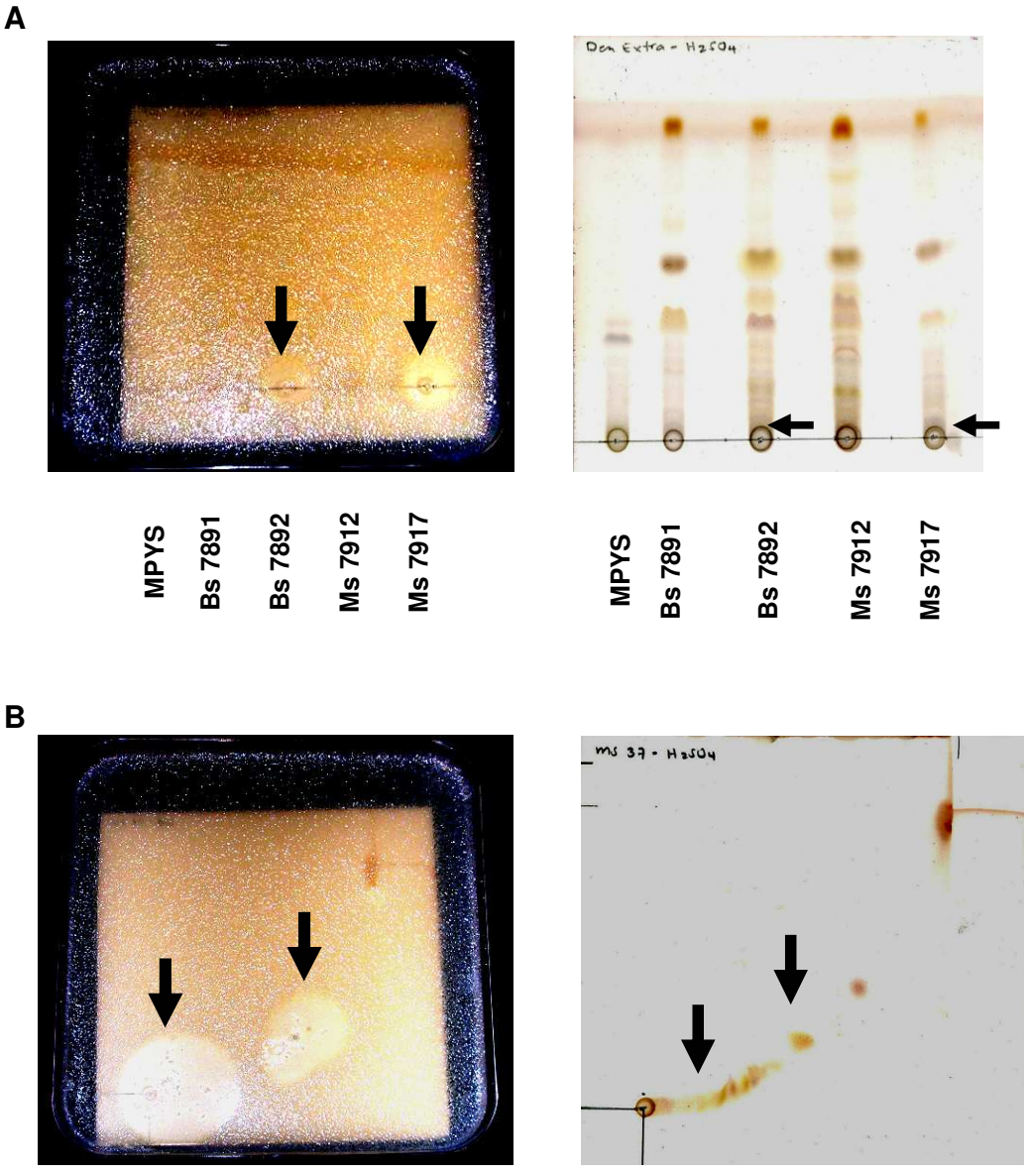
[1] antimicrobial activities as measured by zone of inhibition

0 mm	no inhibition	20 – 30 mm	moderate inhibition
< 20 mm	weak inhibition	> 30 mm	strong inhibition

Zones of inhibition of the uninoculated culture extracts were deducted from the measured zone of inhibition.

[2] geographical origin of strains

Bs – Baltic Sea Ns – North Sea Gm – Gulf of Mexico Ms – Mediterranean Sea
Jp – Japan Fr – France Uk – United Kingdom



Ms 7917 (diameter zone of inhibition = 10 – 31 mm)

Figure 20. Bioautogram of the crude extracts of marine *Dendryphiella* species. TLC plates developed in one direction with 4 % MeOH in dichloromethane (A) and two-dimensionally with 4 % and 8 % MeOH in dichloromethane (B). TLC plates on the right side showed the metabolites as visualized with 5 % H_2SO_4 in EtOH; lightness, contrast and intensity of TLC plate B was modified with Corel Photo-Paint Version 8. Clear zones and possible respective metabolites indicated with black arrows.

IV. Discussion

A polyphasic approach to taxonomy and ecophysiology has been only recently applied to fungi, in contrast to its earlier application to bacteria. It employs an integrated usage of morphological, cultural, biochemical, physiological and molecular characters to analyse species identity, phylogeny and population diversity. Species of the anamorphic fungi *Fusarium* (Yli-Mattila *et al*., 2002) and *Alternaria* (Andersen & Thrane, 1996; Andersen *et al*., 2001, 2002; Pryor & Gilbertson, 2002) as well as the endophytic species of *Leptostroma* (Sieber-Canavesi *et al*., 1991), *Melanconium* (Sieber *et al.,* 1991) and *Xylaria* (Rodrigues *et al*., 1993), for example, have been characterized and differentiated by combined analysis of their morphological, cultural, physiological and molecular characters. In this research study, an integrated, polyphasic approach has likewise been conducted to study the taxonomy and ecophysiology of the marine fungi *D. arenaria* and *D. salina*, thereby providing clear answers to the taxonomic ambiguities and phylogenetic position of these marine fungi as well as insights into their growth and adaptation in the marine habitats.

A. Polyphasic approach to the taxonomy of *D. arenaria* and *D. salina*

The two marine species of *Dendryphiella* have been traditionally identified and differentiated based on their morphological characters. Descriptions of these characters, however, differ and, in case of conidial sizes, overlap between published identification keys, e.g. Ellis (1976), Kohlmeyer & Volkmann-Kohlmeyer (1991) and Hyde & Sarma (2000), thus

rendering it difficult to identify isolated strains of this genus. Michaelis *et al*. (1987) observed such overlapping characters in their population studies of *D. arenaria* and *D. salina*, which led them to question whether two separate species or merely a single species with morphological variations exist. They applied other methods, e.g. ELISA (Mohamad *et al*., 1989), for their differentiation. Strains of *Dendryphiella* isolated in this research study also showed overlapping conidial sizes and morphologies (see Appendix C for the list of strains and their conidial sizes). The isolated *D. arenaria* and *D. salina* strains produced conidia typically observed in the published literature, but their conidial size ranges did not always fit into these descriptions. However, analysis of their mean conidial length and mean colony extension rates on MEAS and PDAS media revealed differences that may be useful in discriminating *D. arenaria* and *D. salina*. Strains of *D. arenaria* had mean conidial lengths shorter (< 20 µm) than those of *D. salina* (Fig. 4). The former also exhibited faster mean colony extension rates (≥ 1.2 cm day^{-1}) than the latter (Fig. 4), a species-specific response observed even under different conditions of culture (dela Cruz *et al*., 2006; Fig. 18).

However, not all isolated strains fitted these observed descriptions, thereby, putting into question the suitability of these characters in the identification of the marine *Dendryphiella*. Six strains (Ms 32, 33, 34, 35, 37, 38) from the Mediterranean Sea, their identity confirmed by gene sequence analysis as *D. salina* (Table 2, Fig. 6 – 8), grew with mean colony extension rates characteristic of *D. salina*, but exhibited mean conidial lengths similar to those of *D. arenaria* (Fig. 4). Thus, conidial morphology and growth rates

alone were not sufficient to completely discriminate *D. arenaria* from *D. salina* and clear taxonomic uncertainties. Similarly, in identifying several species of *Alternaria*, morphology was used successfully only in combination with cultural, biochemical and molecular characteristics (Andersen & Thrane, 1996; Pryor & Gilbertson, 2002). Therefore, to solve the taxonomic uncertainties of these species, a combined genetic and phenotypic analysis was conducted on the isolated strains.

Genetic analysis of the marine *Dendryphiella* species involved multilocus gene sequencing. This typing method uses nucleotide sequences from several genes and was found useful recently in fungal species recognition and population studies (Taylor & Fisher, 2003). However, not all genes can be applied in phylogenomics (Sicheritz-Pontén & Andersson, 2001). Informational genes such as those involved in transcription, translation and related processes were seldomly horizontally transferred (Jain *et al*., 1999), and thus, are more suitable in tracing evolutionary relationships among taxa. Multilocus gene sequence typing was thus employed on *Dendryphiella* strains to determine the existence of either two species or of a single species with morphological variations, but also to determine their taxonomic position in the modern molecular ascomycete phylogeny and their taxonomic relationship to the genus *Scolecobasidium*. *S. arenarium* and *S. salinum* are at present the current valid nomenclature for *D. arenaria* and *D. salina*, respectively, as Ellis (1976), without providing explanation, transferred the two marine species of *Dendryphiella* to the genus *Scolecobasidium*. However, due to the significant physioecological and morphological

dissimilarities between the two genera, this current classification has not been well accepted by other mycologists, raising another ambiguity in the taxonomy of *Dendryphiella*.

RAPD profiles of the *Dendryphiella* species were initially investigated by PCR-amplification of their genomic DNA using the primer M13. RAPD analyses are useful in determining taxonomic diversity within taxa (Guarro *et al*., 1999), though their application at the population level should be done with caution (Bruns *et al*., 1991). Analysis of the RAPD profiles of *D. arenaria* and *D. salina* did not clearly differentiate the two marine species (Fig. 5). No RAPD pattern was observed characteristic of the species in contrast to the taxon- and individual-specific bands found using this method among the toxigenic *Fusarium* species (Altomare *et al*., 1997). This study as well as that of Altomare *et al.* (1997) showed no correlation between geographic origin of the isolates and their RAPD patterns. However, it was clear that both species shared some genetic similarities as shown by the few bands present in most strains of both species (Fig. 5). Since only a single primer (M13) was used to differentiate the marine *Dendryphiella*, it remains to be seen if other RAPD primers could better discriminate the two species as has been done with other fungi, e.g. *Aspergillus* (Yuan *et al*., 1995), *Fusarium* spp. (Altomare *et al*., 1997), *Sclerotinia* (Raina *et al*., 1997), and *Trichoderma* (Wuczkowski *et al*., 2003). The use of a single primer, here, was justified since it was not the intention to provide a thorough study of the genetic variability between *D. arenaria* and *D. salina* strains with this method, but rather to use the RAPD profiles for delimiting strains for subsequent gene sequencing.

The *rpb*2 gene which encodes for the second largest RNA polymerase subunit, was first sequenced to provide better resolution between the species of *Scolecobasidium* and *Dendryphiella*, thereby determining the generic affiliation of *D. arenaria* and *D. salina* and eventually, their taxonomic position in the modern phylogeny of ascomycetes. The nuclear *rpb*2 gene has been applied in establishing the phylogenetic relationships among ascomycetes and has additional useful properties of being single copy and having a relatively slow evolutionary rate (Liu *et al*., 1999; Liu & Hall, 2004). Analysis of the *rpb*2 gene sequence of the marine *Dendryphiella* species showed two distinct sister clades for *D. arenaria* and *D. salina* (Fig. 6). The representative terrestrial *Dendryphiella* species, *D. vinosa* NBRC 32669 and *Dendryphiella* sp. NBRC 100153, however, grouped separately from the marine strains. All *Dendryphiella* species, though, grouped with the family *Pleosporaceae*. The distant clustering of the marine and terrestrial *Dendryphiella* was not unexpected since the genus was said to be polyphyletic (E. B. G. Jones, pers. comm., 06 Nov. 2005). The taxonomic grouping of the different species of *Dendryphiella* into one genus was mainly based on their morphological similarities, as these characters were previously those primarily used in the discrimination of anamorphic fungal genera.

Interestingly, analysis of the *rpb*2 gene sequence also revealed that the representative strains of *Scolecobasidium* formed a phylogenetic group outside of the class *Loculoascomycetes* and were clearly distant from both the terrestrial and marine species of *Dendryphiella* (Fig. 6). Analysis of the conidial formation likewise revealed differences between the two genera.

Scolecobasidium is characterized by polyblastic, denticulate conidiogenous cells with long, narrow-cylindrical, threadlike denticles which often break across the middle leaving part of it attached to the conidium and part to the conidigenous cells, whereas *Dendryphiella* has polytretic, cicatrized conidiogenous cells without denticles (Ellis, 1971). The isolated *Dendryphiella* strains in this research study likewise showed the absence of denticles as similarly illustrated by Pugh & Nicot (1964) and Kohlmeyer & Kohlmeyer (1979). Though the phylogenetic position of *Scolecobasidium* remained unclear, this research study clearly concluded that *D. arenaria* and *D. salina* are not congeneric with the species of *Scolecobasidium* and proposed the return to the former nomenclature for their valid scientific names.

The marine *Dendryphiella* species were also sequenced for their internal transcribed spacer region 1 and 2 (ITS 1 and 2) and the large intron of the translation elongation factor 1-alpha (*tef*1) genes to analyze their inter- and intra-species similarities. These genes are sufficiently variable to discriminate between taxa from classes to species level (Mitchell *et al*., 1995; Druzhinina, unpubl.) and thus, were considered for determining the infragenic structure of both the marine and terrestrial *Dendryphiella*. Sequence analysis of these genes similarly resulted in two distinct sister clades which corresponded to *D. arenaria* and *D. salina*, irrespective of their geographic origin and were again separated from the terrestrial strains (Fig. 7 & 8). The clades *D. arenaria* and *D. salina* were also closely related to the genus *Pleospora* (anamorph *Stemphylium*) of the family *Pleosporaceae*, their

phylogeny likewise established based on ITS and glyceraldehyde-3-phosphate dehydrogenase sequence data (Câmara *et al*., 2002). The two sister clades of marine *Dendryphiella*, though distinct, were very close to each other, differing only in 15 nucleotides in their ITS gene sequences and 10 nucleotides in their *tef*1 gene sequences. Isolates with ITS sequences that differ by two or more nucleotides were previously considered to be distinct species in the *Mycosphaerella* clade (Goodwin *et al*., 2001). Following the multiloci phylogenetic analysis, the existence of two distinct species of marine *Dendryphiella*, *D. arenaria* and *D. salina*, was therefore concluded in accordance with the genealogical concordance phylogenetic species recognition concept of John Taylor (Taylor *et al*., 2000). The existence of these two marine *Dendryphiella* species was likewise supported by their phenotypic analysis.

Phenotypic analysis of the *Dendryphiella* species involved investigations on their ability to utilize various substrates and to produce extracellular enzymes and secondary metabolites *in vitro* in order to determine traits that can be useful in discriminating the two species. Mohamad *et al*. (1989) initially employed ELISA and was found to be sensitive in detecting anti-*D. arenaria* and anti-*D. salina* antibodies. In this research study, the ability of *Dendryphiella* to utilize various substrates was investigated using the BIOLOG Phenotype Microarray and their growth on various algal components in microtiter plates. The ability to produce extracellular enzymes was detected using the conventional solid culture media and the semi-quantitative API ZYM assay, whereas the secondary

metabolic profiles were detected with TLC and HPLC-DAD using the culture ethylacetate extracts of selected strains. Substrate utilization and enzyme profiles have been successfully employed in differentiating species of *Trichoderma* (Grondona *et al*., 1997; Kubicek *et al*., 2003), *Beauveria* and *Typocladium* (Todorova *et al*., 1998) as well as *Penicillium* (Bridge & Hawksworth, 1984; Jimenez *et al*., 1990; Seifert *et al*., 2000). Secondary metabolites also has been found stable, easy to reproduce and more objective than morphological data (Frisvad, 1989; Frisvad *et al*., 1998), and thus, are equally efficient for identification and classification of fungi (Smedsgaard & Nielsen, 2005).

Analysis of the BIOLOG Phenotype Microarray revealed several substrates useful in discriminating the two marine species of *Dendryphiella*, i.e. D-mannitol, D-sorbitol, *myo*-inositol, xylitol, arbutin, L-rhamnose and quinic acid (Fig. 11, 12), and principal component analysis of BIOLOG substrate utilization resulted in two separate groups (Fig. 13). The BIOLOG PM could likewise differentiate sub-populations within *Dendryphiella* species corresponding to their geographic origin and/or growth rate (Fig. 9). An interesting observation was the clustering of all *D. salina* strains isolated from the European Atlantic Coast and of the sub-clustering of *D. salina* strains from the Mediterranean Sea and *D. arenaria* strains from the Baltic Sea, correlating with their growth rates on several carbon sources (Fig. 9).

However, not all phenotypic characters studied were found useful in differentiating the two species. The abilities of *D. arenaria* and *D. salina* to produce extracellular enzymes and to utilize algal components as substrates

were similar (Table 5, 6 & 7), though the amounts of enzymes detected using the semi-quatitative API ZYM enzyme assay varied between species and strains. The high general similarities of the two species with respect to their extracellular enzyme profiles and their abilities to utilize similar substrata, characterized by their high simple matching coefficient values (Table 4), may indicate an overlapping continuum of carbon utilization properties of *D. arenaria* and *D. salina* and may reflect the fact that these two species inhabit the same ecological niche. Since the *D. arenaria* and *D. salina* species are not easy to distinguish by morphological analysis, the BIOLOG PM may offer a fast and reliable test for laboratories which do not have facilities for DNA work.

The secondary metabolic profiles of the crude culture extracts of *D. arenaria* and *D. salina* were also studied. Chemical analysis of phytotoxins, mycotoxins and other related secondary metabolites, once standardized, can be more objective than morphological characters and discriminating as exemplified in species of *Penicillium*, *Aspergillus*, *Fusarium* (Filtenborg *et al*., 1983; Frisvad, 1989), *Phoma* (Pedras & Biesenthal, 2000; Osterhage *et al*., 2000) and *Pezicula* (Schulz *et al*., 1995). Metabolic profiles can be used to identify certain genera; for example fusaric acid and the enniatins are characteristic metabolites of *Fusarium* spp. (Turner & Aldridge, 1983). However, the metabolites are not necessarily specific for the genus, e.g. *Gnomonia erabunda* also produces enniatins (Turner & Aldridge, 1983). Other metabolites can differentiate species of a genus. Mellein and cryptosporiopsin were synthesized by various species of *Pezicula* (anamorph

Cryptosporiopsis), but mycorrhizin only by *Pezicula livida* and *P. carpinea* (Schulz *et al.*, 1995). However, in this research study, the application of the secondary metabolic profiles was problematic when used in discriminating the two *Dendryphiella* species. Both the HPLC-DAD (Fig. 16) and the thin-layer chromatograms (Fig. 14) of the ethylacetate crude extracts from cultures of *D. arenaria* and *D. salina* revealed similar metabolic profiles regardless of the origin of the strains. Variations in secondary metabolite production are often observed between different isolates of same species from different habitats and geographical locations (Wildman, 1995; Talbot *et al.*, 1996), but this was not the case for the marine *Dendryphiella*. Following visualisation of the TLC's with Cer-reagent and $FeCl_3$ in HCl to reveal triterpenes and phenolic metabolites, only culture extracts of five strains of *D. salina* were found to produce these metabolites (Fig. 15). Thus, they too are not useful for differentiation. And since both species occupy the same ecological niche, and perhaps are threatened by similar microbial competitors, production of similar metabolites was expected. Differences in the amount of metabolites produced, however, could be observed on the chromatogram as indicated by the heights of the different peaks.

To date, the only metabolites that have been detected in culture extracts of *D. salina* were dendryphiellin A – D and A1, the first fungal trinor-eremophilanes, three eremophilanes (Dendryphiellin E, F and G), the C9-carboxylic acids, dendryphiellic acids A and B, a novel glyceryl ester (glyceryl dendryphiellate A), and the eremophilanes (Dendryphiellin E1 and E2) (Guerriero *et al.*, 1988, 1989, 1990). But since these reference substances

were not available, it was not possible to check if any of the metabolites detected corresponds to those substances.

B. Polyphasic approach to the ecophysiology of *D. arenaria* and *D. salina*

Substrate utilisation profiles from BIOLOG FF MicroPlates and API 50 CH biochemical tests and enzyme profiles from API ZYM were previously applied to assess functional diversity and nutritional profiles of individual fungi (Dobranic & Zak, 1999; Tanzer *et al*., 2003), as well as in the analysis of fungal communities (Buyer *et al*., 2001) and microbial bioprocesses (Tiquia, 2002). In this research study, the assimilation of carbon sources by the two marine species of *Dendryphiella*, *D. arenaria* and *D. salina*, was investigated not only to discriminate the two species but also to learn to what extent these fungi use a broad range of carbon sources. Of note is the fact that all isolates utilize a broad range of carbon sources. This may contribute to their phenotypic plasticity, being one of the reasons (dela Cruz *et al*., 2006) why these species can be found on varied substrates in different marine habitats and climate zones.

The BIOLOG PM data showed that carbon preference of the two fungi differed in several respects from that of the terrestrial ascomycetes thus far investigated, e.g. *Aspergillus nidulans* (Tanzer *et al*., 2003) and *Hypocrea jecorina* (Druzhinina *et al*., 2006). One of the most obvious findings was the observation that both species grew poorly on D-arabitol and glycerol (Fig. 11). *D. arenaria* also grew poorly on i-erythritol (Fig. 11) and did not utilize D-mannitol and D-sorbitol, though these substrates allowed good growth of *D.*

salina strains (Fig. 12). This poor growth on polyols, which are good carbon sources allowing faster growth of other ascomycetes (Tanzer *et al*., 2003; Druzhinina *et al*., 2006), contrasts with the fact that marine *Dendryphiella* spp. accumulate some of these polyols as a means of osmotic adjustment to counteract salinity stress in their natural habitats (Wethered *et al*., 1985; Clipson *et al*., 1990), and therefore, ought to have the enzymes necessary for their synthesis and catabolism. The present data, however, imply that *Dendryphiella* either has no uptake mechanisms for polyols or controls them by other factors than availability in the medium. This finding, therefore, indicates that adaptation to their habitat has been accompanied by changes in polyol metabolism.

Another interesting finding is the poor growth on or inability to utilize N-acetylglucosamine and D-glucosamine (Fig. 11), both of which are generally well utilized by terrestrial ascomycetes and contrasts to the detection of the constitutive N-acetylglucosaminidase activities found in all strains (Table 7). This may indicate that the latter serves only for turn-over of the cell wall during growth of *Dendryphiella* and not for their utilization of other chitin-containing substrates, e.g. other fungi or insects.

On the other hand, this study also detected some substrates which are generally considered to be poor substrates for fungi, e.g. succinamide and α-1,3-glucosyl-fructoside (turanose); both were utilized at high rates by the two species of *Dendryphiella*. Succinamide is hydrolysed by a nitrilase, which is apparently absent from most other fungi in contrast to succinate which is utilized by all fungi (Nawaz *et al*., 1989; Linardi *et al*., 1996). Turanose, on

the other hand, serves as a substrate for some but not all α-glucosidases (Suzuki *et al*., 1997). α-glucosidase was detected using the API ZYM assay in all strains (Table 7). This ability of marine *Dendryphiella* opens a potential application for obtaining biocatalysts with new properties.

In order for marine fungi to survive, they must possess the enzymes to degrade the organic compounds present in their native habitat. The strains of *Dendryphiella salina* and *D. arenaria* studied were able to utilize various sugars and complex organic compounds, but also an extract of *Laminaria digitata* and laminarin, the stored polysaccharide common in *Laminaria* (Table 5). Degradation of laminarin (Schatz 1980, 1984; Grant & Rhodes, 1992) and alginate (Wainwright, 1980; Wainwright & Sherbrock-Cox, 1981; Schaumann & Weide, 1990, 1995) were also previously reported, however in contrast to this study only for several isolates each. The isolates, however, were not able to degrade fucoidan. Of note: A number of the *Dendryphiella* strains were isolated from *Laminaria digitata*, but few from *Fucus* spp. (unpublished), and molecular studies failed to identify *Dendryphiella* spp. associated with the latter (Zuccaro *et al*., 2003).That *Dendryphiella* species are encountered on moribund or decayed algal debris may be merely coincidental to the ease of finding these materials washed up ashore during low tide. It is probable that their spectrum of substrate preference is wider than is presently known. Their large arsenal of degradative enzymes suggests that they also have a parasitic potential. There is but a thin blurred line between facultative parasitism, saprophytism and “endophytism”, as is known for many pathogenic fungi on terrestrial plants (Schulz & Boyle, 2005).

The ability to utilize carbon sources and algal components as detected by the BIOLOG phenotype microarrays and microtiter plate assay was generally concordant with the results obtained from analysing extracellular enzymes (Table 6 and 7), e.g. all 51 strains of *D. arenaria* and *D. salina* produced the hemi-cellulolytic enzyme xylanase and the extracellular enzymes amylase, lipase and urease, thereby confirming earlier studies done for several strains of *D. salina* (Gessner, 1980; Fenice *et al*., 1997). The ability to degrade storage products and structural components of plants such as hemicellulose, starch and lipids appears to be a common characteristic of the marine *Dendryphiella* species, which is consistent with the isolation of several strains in this study from *Zostera marina*, a marine plant. Although all the isolates of both species produced the extracellular cellulolytic enzyme β-glucosidase, the cellulase activitiy was below the detection limit. This is in contrast to results reported by MacDonald & Speedie (1982) for *D. arenaria,* who found cell-associated and extracellular cellulolytic enzymes, and to those of Rohrmann & Molitoris (1992), who found a strong cellulase activity in two strains of *D. salina.* Since *Dendryphiella* species have been commonly isolated from decaying plants, they ought to be able to produce the cellulolytic enzymes needed for degradation of the plants' cell walls. Perhaps the assay used in this study is not sufficiently sensitive.

The *Dendryphiella* isolates also exhibited similar API ZYM profiles, irrespective of species or geographical origin (Table 7), only differing in the amounts of substrates hydrolysed. Generally, both species produced extensively the enzymes β-glucosidase and N-acetyl-β-glucosaminidase,

though most strains of *D. salina* produced larger amounts of α-galactosidase. The similarity in extracellular enzyme profiles observed for isolates of *D. arenaria* and *D. salina* could be attributed to the similar substrates the two species utilise *in situ*.

This research study also revealed numerous adaptations to the abiotic and biotic parameters of the marine environment. The salinity and temperature ranges of the seas from which the isolates originated varied considerably (Table 3). Nevertheles, all of the isolates were equally adapted to grow under all the conditions tested, demonstrating their phenotypic plasticity and the ability of each isolate to adapt to diverse biotopes. Additionally, there were no correlations of biological activity of the culture extracts with geographical locations; though production of bioactive metabolites was strain-specific.

Salinity has always been recognized as a key determinant factor for the growth of marine fungi. However, it is not an absolute requirement as many marine fungi including several strains of *D. salina* have been found to grow in culture both without added salts and even well with minimal amounts of added salts (Jones & Jennings, 1964; Jones & Byrne, 1976). Similarly, in this study, absence of artificial marine salts in culture media did not prevent growth of any of the isolates of *D. arenaria* and *D. salina*, though there were significant differences in colony extension rates between those cultured with and without marine salts (Fig. 18.A). The isolates were not only able to adapt to low marine salt concentrations, but also to high concentrations, e.g. the good growth of the Baltic Sea isolates at the highest marine salt

concentration tested (45 g L^{-1}), somewhat surprising since the coastal waters of the Baltic Sea have a low salinity (approx. 10 g L^{-1};Table 3). Adaptations to salinity require the fungi to accumulate high concentrations of intracellular solutes to ensure that water will enter and not leave the cell (Clipson & Jennings, 1992). To maintain this negative cellular water potential *vis-à-vis* its external environment, *D. salina* can increase cytoplasmic and vacuolar volume (Clipson *et al*., 1989), though osmotic adjustment in the cytoplasm is likely maintained by synthesis of organic solutes such as mannitol, glycerol and arbitol (Wethered *et al*., 1985; Clipson *et al*., 1990).

Temperature also plays an important role in the growth of fungi. The strains of *D. arenaria* and *D. salina* from various geographical locations exhibited in culture a broad temperature range for growth (Fig. 18.B) – from cool (5 – 22 °C) to warm (25 – 34 °C) – although the growth optimum was at 25 °C, even for isolates from the Baltic and North Seas where even in summer the temperature is not higher than 20 °C (Table 3). Spore formation occurred between 15 and 34°C. The results obtained in these investigations with many isolates from different climates nevertheless concur with those of Duffy *et al*. (1991), Panebianco (1994) and Jones & Byrne (1976), who studied fewer isolates from only one climatic zone. Hughes (1974) and Kohlmeyer (1983) asserted that temperature may be the limiting factor that determines fungal geographical distribution. Perhaps it is the plasticity to grow and survive at a broad range of temperatures as demonstrated by the 16 strains tested that enables the marine *Dendryphiella* species to grow in coastal areas of the tropical, sub-tropical and temperate zones.

The "*Phoma*-pattern of growth" describes an adaptation of a number of fungi to growth in the coastal habitat in marine environments and appears not to be correlated with any taxonomical group or geographical distribution (Lorenz & Molitoris, 1992). All of the 16 strains of *Dendryphiella*, again irrespective of origin, exhibited this pattern of growth (Fig. 19) as had been found by Lorenz & Molitoris (1992) for three temperate strains of *D. salina*, suggesting that this is a general characteristic of the marine *Dendryphiella* species. Lorenz & Molitoris (1992) argued that along intertidal zones, water recedes at low tide and the little pools of water that remain heat up, resulting in increased salinity due to evaporation. Therefore, marine fungi that can tolerate higher salinities at higher temperatures have a survival advantage, another example of the phenotypic plasticity that these marine species of *Dendryphiella* exhibit.

This study also showed only slight differences in the growth of *D. salina* and *D. arenaria* at the pH range (5.0 – 8.0) tested irrespective of their origin (Fig. 18.C) as also previously reported by Curran (1980) and Edwards *et al.* (1998). The capability to grow at higher pH-values, which is not common among fungi, suggests though an adaptation to the alkalinity of the marine habitat (Edwards *et al.*, 1998).

Dendryphiella spp. as saprobic fungi have numerous other microorganisms as competitors for the degradation of organic matter. Production of bioactive metabolites would enhance their chances to colonize and degrade substrates. In contrast to previous results (Pugh, 1974; Otsuka *et al.*, 1992), all 22 of the tested strains of *D. arenaria* and *D. salina* produced

antifungal metabolites, many synthesized antibacterial and antialgal metabolites. Such substances could give them an advantage over microbial competitors (Table 9 & 10). The antialgal substances might also be advantageous in dealing with the algal defense response, since analogous to plants, macroalgae use chemical defense against invading microorganisms (Kubanek *et al.*, 2003). Inhibition of *E. coli* was only weak to nil, perhaps because this bacterium is allochthonous in the marine environment.

Bioactivity against the test microorganisms appeared to be strain-specific, e.g. only seven strains of *D. salina* (Bs 7892, Ms 7915, Ms 7917, Ns 7908, Ns 7909, Uk 8221, Uk 8829) exhibited moderate (20 – 30 mm) to strong (> 30 mm) inhibition of the test fungus *M. violaceum*. Inhibition was also sometimes dependent on the culture medium and incubation temperature (see Appendix E.2 for the biological activities of the individual *Dendryphiella* strains), as is frequently the case for the fungal synthesis of secondary metabolites (Frank, 1998). Again, bioactivities did not correlate with geographical origin of the strains.

TLC detected triterpenes and phenolic metabolites from the crude culture extracts of marine *Dendryphiella* (Fig. 15). But the intensity of the bands seems not correlated with antifungal activity (Table 10), suggesting that triterpenes and phenolic metabolites are not responsible for the inhibitions. Similarly, the antifungal, hydrophobic metabolite ergosterol, which was found in the culture extracts of *D. arenaria* (Dai and Krohn, unpubl., Lösgen and Zeeck, unpubl.) cannot be primarily responsible for the strong inhibitions, since the antifungal metabolites are relatively polar, remaining at

the origin with 4 % methanol in dichloromethane as solvent, c.f. Fig. 20. The known metabolites from the culture extracts of *D. salina* were dendryphiellin A – G, dendryphiellic acids A and B, and glyceryl dendryphiellate A (Guerriero *et al*., 1988, 1989, 1990). The authors reported no antifungal properties of these metabolites. And since these reference substances were not available, it could not be checked if the bioactive secondary metabolites found in these investigations are similar to those identified by Guerriero *et al*. (1988, 1989, 1990).

In summary, the polyphasic approach employed in this research study provided a better understanding of the taxonomy and ecophysiology of the marine *Dendryphiella*. The genetic and phenotypic profiles clearly differentiated *D. arenaria* from *D. salina*, as well as the two marine species from other terrestrial *Dendryphiella* species, though some phenotypic characters were found not to be useful in discriminating the two species. However, the analysis likewise provided evidence for misplacement of marine *Dendryphiella* species in the genus *Scolecobasidium* and supported the usage of its former nomenclature, *D. arenaria* and *D. salina*, as its valid taxonomical name. This research study also deepened our understanding of the physiological adaptations of these ecologically important fungi to their natural marine niches.

V. Summary

The marine fungi *Dendryphiella*, *D. arenaria* and *D. salina*, were investigated for their genotypic and phenotypic characteristics in order to determine their taxonomic position with respect to the modern molecular phylogeny of ascomycetes, but also their physiological responses in their natural habitat. Fifty-seven strains of *Dendyphiella* from different geographical locations and climatic zones were either isolated following surface-sterilization of decaying or living algal and plant materials or obtained from culture collections, grown as axenic, monospore cultures and initially identified according to spore morphology as *D. arenaria* and *D. salina*. Subsequent identification on the basis of their genetic and phenotypic characteristics resulted in 22 strains of *D. arenaria* and 35 strains of *D. salina*.

Whereas most of the isolates of the two species differed significantly in their conidial morphology and mean colony extension rates, e.g. strains of *D. salina* had longer conidia (≥ 20 µm) than those of *D. arenaria*, but with the latter having faster mean colony extension rates (≥ 1.2 cm day $^{-1}$) than the former, these characteristics did not fit all of the isolated strains, and thus, were insufficient when used alone in discriminating the two species.

Analysis of their ITS 1 and 2, *tef*1 and *rpb*2 gene sequences fully differentiated the marine *Dendryphiella* and clearly supported the existence of two very closely related species. Strains of *D. arenaria* and *D. salina* all grouped in the family *Pleosporaceae*, with *Pleospora* spp. (anamorph *Stemphylium* spp.) as their next closest taxonomic relative. The

Scolecobasidium species sequenced formed a distinct and genetically isolated phylogenetic group outside of the class *Loculoascomycetes*, and thus, were not congeneric with *Dendryphiella*. The nomenclature *D. arenaria* and *D. salina* should therefore be used for valid taxonomy of the species.

Analysis of the phenotypic profiles of the two *Dendryphiella* species revealed similar extracellular enzyme profiles using conventional cultural methods and by semi-quantitative API ZYM assay, though the amount of enzymes detected varied between strains. *D. arenaria* and *D. salina* produced amylase, xylanase, α- and β-glucosidase, α-galactosidase, N-acetylglucosaminidase, esterase, lipase, polyphenol oxidase and urease, though laccase and peroxidase activities were confined to only some strains. On the other hand, the carbon utilization profiles as determined by the BIOLOG Phenotype Microarrays (PM) differed significantly, e.g. in their utilization of quinic acid, D-mannitol, D-sorbitol and xylitol. Utilization of these substrata was useful in discriminating not only the two species, but also sub-populations within *Dendryphiella* species in relation to their geographic origin and/or growth rate. BIOLOG PM also showed abilities of marine *Dendryphiella* to utilize substrata such as succinamide and turanose, which differentiated them from other terrestrial ascomycetes.

Detection of their secondary metabolites from crude culture extracts with TLC and HPLC-DAD also revealed that strains of the two species had similar profiles. Triterpenes and phenolic compounds were only detected on the TLC's of five strains of *D. salina*.

Physiological responses to abiotic and biotic factors by the *Dendryphiella* strains showed phenotypic plasticity, implying their adaptive capability to grow on various substrates, in varied geographical locations and climatic zones. All tested strains of *D. arenaria* and *D. salina* grew under all conditions tested, but optimally on culture media with added marine salts, at pH values 6.5 – 8.0 and at an incubation temperature of 25 ^{o}C. All strains exhibited an increased salt optimum with increasing incubation temperature and utilized various algal components and algal extracts. The culture extracts of the tested strains were also antimicrobial, though production of the biologically active metabolites was strain-specific. The identity of the bioactive secondary metabolites, however, remained unknown.

V. Zusammenfassung

Die marinen Arten der Gattung *Dendryphiella*, *D. arenaria* und *D. salina*, wurden auf ihre genotypischen und phänotypischen Unterschiede hin untersucht, um die taxonomische Position der *Dendryphiella*-Spezies in Bezug auf die moderne Phylogenie von Ascomyceten, und das Wachstum und die Adaptation der beiden Spezies an den marinen Lebensraum zu klären. Siebenundfünfzig Stämme von *D. arenaria* und *D. salina* aus verschiedenen geographischen Lagen und Klimazonen wurden entweder aus marinen Algen und Pflanzen isoliert oder von Stammsammlungen bezogen. Die *Dendryphiella*-Spezies wurden als axenische, monospore Kulturen kultiviert und aufgrund ihrer Konidienmorphologie als *D. arenaria* und *D. salina* identifiziert. Nachfolgende Identifizierung von genetischen und phänotypischen Eigenschaften ergab 22 Stämme von *D. arenaria* und 35 Stämme von *D. salina*.

Es gab Unterschiede bei der Konidienmorphologie und den Wachstumsraten der zwei Spezies, z.B. die meisten Stämme von *D. salina* hatten längere Konidien (≥ 20 µm) als die Stämme von *D. arenaria*, aber letztere wuchsen meist schneller (≥ 1.2 cm day $^{-1}$) als erstere. Die Isolate stimmten jedoch mit diesen Merkmalen nicht immer überein. Deswegen waren die morphologischen und kulturellen Charaktere nicht ausreichend um *D. arenaria* von *D. salina* zu differenzieren.

Die Sequenzanalyse von ITS 1 und 2, *tef*1 und des *rpb*2-Gens differenzierte jedoch die marinen *Dendryphiella-Stämme* und erhärtete die Annahme der Existenz von zwei distinkten aber nahe verwandte Spezies, die

D. arenaria und *D. salina* entsprechen. Ebenso zeigte auch die Gensequenzanalyse, dass *D. arenaria* und *D. salina* in die Familie der *Pleosporaceae* gehören und dass *Pleospora* spp. (anamorph *Stemphylium*) das am nächsten verwandte Taxon ist. Die *Scolecobasidium*-Spezies, die auch sequenziert wurden, bildeten eine genetisch und phylogenetisch distinkte Gruppe, die außerhalb der Klasse *Loculoascomyceten* steht. Das zeigt, dass *D. arenaria* und *D. salina* nicht zu dieser Gattung gehören und nicht *S. arenarium* und *S. salinum* genannt werden sollten. Die Namen *D. arenaria* und *D. salina* sollten daher für die gültige Taxonomie für diese Spezies verwendet werden.

Analysen der phänotypischen Profile von Isolaten der beiden *Dendryphiella*-Arten zeigten Ähnlichkeiten Ihrer Enzymprofile, bestimmt durch Methoden der Kultivierung und durch API ZYM-Tests, obwohl die Mengen der Enzyme, die produziert wurden, variabel waren. *D. arenaria* und *D. salina* produzierten die extrazellulären Enzyme Amylase, Xylanase, α- und β-Glucosidase, α-Galactosidase, N-Acetylglucosaminidase, Esterase, Lipase, Polyphenol Oxidase und Urease. Laccase und Peroxidase werden nur bei einigen Isolaten produziert. Anderseits unterschieden sich die Substratabbauprofile mit BIOLOG Phenotype Microarrays (PM) bei den zwei Spezies und sogar bei den Subpopulationen in Bezug auf ihren geographischen Ursprung und/oder die Wachstumrate. Einige C-Quellen, z. B. Chinin-Säure, D-Mannit, D-Sorbit und Xylit können für die Differenzierung der *D. arenaria* von *D. salina* benützt werden. Substrate wie Succinamide und Turanose wurden von marinen *Dendryphiella*-Stämmen abgebaut, aber

nicht von anderen terrestrischen Ascomyceten und können sogar als Unterscheidungsmerkmal zwischen marinen und terrestrischen Pilzen genützt werden.

Detektion der Sekundärmetabolite ihrer Kulturextrakte mit TLC und HPLC-DAD ergaben auch ähnliche Profile. Triterpene und phenolische Substanzen, die nur bei fünf Stämmen von *D. salina* auftraten, wurden mit TLC detektiert.

Physiologische Reaktionen der *Dendryphiella*-Stämme auf abiotische und biotische Einflüsse zeigten phänotypische Plastizität, die diesen Organismen auf verschiedenen Substraten, geographischen Lagen und Klimazonen die Fähigkeit zu wachsen geben. Alle getestete Stämme der *D. arenaria* und *D. salina* wuchsen bei allen getestete Kulturbedingungen, aber optimal auf Kulturmedien mit Meersalz, bei pH-Werten 6.5 – 8.0 und bei der Inkubationstemperatur 25 °C. Die Stämme zeigten ein erhöhtes Salzoptimum bei erhöhten Inkubationstemperaturen und bauten verschiedene Algenkomponenten und Algenextrakte ab. Die Rohkulturextrakte der Isolate waren antimikrobiell, die Produktion der einzelnen bioaktiven Metabolite war jedoch stammspezifisch. Die Identitäten dieser bioaktiven Metabolite sind noch unbekannt.

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Appendix A

Geographic Origin of the Strains of *D. arenaria* and *D. salina*

Strain Nr. (TUBs)	Taxon	Substrate	Surface-sterilization [a]	Date of Isolation	Geographical Origin
7888	*D. arenaria*	*Ceramium* sp.	1	Sept. 2003	Rügen Island, Germany (Baltic Sea)
7889	*D. arenaria*	*Fucus* sp.	2		
7890	*D. arenaria*	*Fucus* sp.	2		
7891	*D. arenaria*	*Polysiphonia urceolata*	2	Nov. 2002	Friedrichsort, Germany (Baltic Sea)
7892	*D. salina*	*P. urceolata*	2		
7893	*D. salina*	*Laminaria digitata*	1	Aug. 2003	Helgoland, Germany (North Sea)
7894	*D. salina*	*L. digitata*	1		
7895	*D. salina*	*L. digitata*	1		
7896	*D. salina*	*L. digitata*	3		
7897	*D. salina*	*L. digitata*	3		
7898	*D. salina*	*L. digitata*	3		
7899	*D. salina*	*L. digitata*	3		
7900	*D. salina*	*L. digitata*	3		
7901	*D. salina*	*L. digitata*	3		
7902	*D. salina*	*L. digitata*	3		
7903	*D. salina*	*L. digitata*	1		
7904	*D. salina*	*Fucus serratus*	1		
7905	*D. salina*	*F. serratus*	1		
7906	*D. salina*	*F. serratus*	1		
7907	*D. salina*	*L. digitata*	1		
7908	*D. salina*	*L. digitata*	3		
7538	*D. arenaria*	*Hypnea musciformis*	4	Oct. 2003	John's Pass Florida, USA (Gulf of Mexico)
7527	*D. arenaria*	*Digenea simplex*	4		
7479	*D. arenaria*	*Gracillaria tikvalriae*	5	Oct. 2003	Fort de Soto Florida, USA

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Geographic Origin of the Strains of *D. arenaria* and *D. salina*

Strain Nr. (TUBs)	Taxon	Substrate	Surface-sterilization [a]	Date of Isolation	Geographical Origin
7508	*D. salina*	*Ceramium* sp.	4	Oct. 2003	Fort de Soto Florida, USA (Gulf of Mexico)
7515	*D. arenaria*	n. d.	5		
7541	*D. arenaria*	*Gracillaria* sp.	5	Oct. 2003	John's Pass, Fl.
6551	*D. arenaria*	*Sargassum* sp.	5	Apr. 2002	Gulf of Mexico
7909	*D. salina*	*Glaux maritima*	5	Aug. 2003	Cuxhaven, Germany (North Sea)
7910	*D. salina*	n. d.	5		
7911	*D. arenaria*	n. d.	5	n. d.	Crete, Greece (Mediterranean)
7912	*D. salina*	n. d.	5		
7913	*D. salina*	n. d.	5		
7914	*D. salina*	n. d.	5		
7915	*D. salina*	n. d.	5	n. d.	Gozo, Malta (Mediterranean)
7916	*D. arenaria*	n. d.	5		
7917	*D. salina*	n. d.	5		
7918	*D. salina*	n. d.	5		
7919	*D. arenaria*	n. d.	5		
7920	*D. salina*	n. d.	5		
7921	*D. salina*	*L. digitata*	3	Aug. 2003	Helgoland, Germany (North Sea)
7922	*D. salina*	*L. digitata*	3		
7923	*D. salina*	*L. digitata*	3		
7924	*D. salina*	*L. digitata*	3		
7925	*D. salina*	*F. serratus*	1		
8147	*D. salina*	*Enteromorpha intestinalis*	5	Sept. 2004	France, Atlantic
8194	*D. arenaria*	*Sargassum* sp.	1	Oct. 2004	John's Pass Florida, USA
8195	*D. arenaria*	*Sargassum* sp.	1		

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Geographic Origin of the Strains of *D. arenaria* and *D. salina*

Strain Nr. (TUBs)	Taxon	Substrate	Surface-sterilization [a]	Date of Isolation	Geographical Origin
8196	*D. arenaria*	*Sargassum* sp.	2	Oct. 2004	John's Pass Florida, USA (Gulf of Mexico)
8197	*D. arenaria*	*Sargassum* sp.	2		
8198	*D. arenaria*	*Sargassum* sp.	2		
8199	*D. arenaria*	*Sargassum* sp.	2		
8200	*D. arenaria*	*Sargassum* sp.	1		
8201	*D. arenaria*	*Sargassum* sp.	1		
8202	*D. arenaria*	*Sargassum* sp.	1		
8203	*D. arenaria*	*Sargassum* sp.	1		
8204	*D. arenaria*	*Sargassum* sp.	1		
8205	*D. arenaria*	*Sargassum* sp.	1		
8206	*D. arenaria*	*Sargassum* sp.	1		
8207	*D. arenaria*	*Sargassum* sp.	2		
8208	*D. arenaria*	*Sargassum* sp.	2		
8210	*D. arenaria*	*Sargassum* sp.	2		
8213	*D. arenaria*	*Zostera marina*	1		
8214	*D. arenaria*	*Z. marina*	1		
8215	*D. arenaria*	*Z. marina*	1		
8216	*D. arenaria*	*Z. marina*	1		
8217	*D. arenaria*	*Z. marina*	2		
8218	*D. arenaria*	*Z. marina*	2		
8219	*D. salina*	*Fucus* sp.	4	n. d.	Lulworth Coast, United Kingdom (English Channel)
8220	*D. salina*	*Fucus* sp.	4		
8221	*D. salina*	*Fucus* sp.	4		
8222	*D. salina*	*F. serratus*	4		

Appendix A

Geographic Origin of the Strains of *D. arenaria* and *D. salina*

Strain Nr. (TUBs)	Taxon	Substrate	Surface-sterilization [a]	Date of Isolation	Geographical Origin
8223	*D. salina*	*F. serratus*	4	n. d.	Lulworth Coast, United Kingdom (English Channel)
8224	*D. salina*	*F. serratus*	4		
8225	*D. salina*	*Fucus vesiculosus*	4		
8226	*D. salina*	*F. vesiculosus*	4		
8227	*D. salina*	*F. vesiculosus*	4		
8228	*D. salina*	*F. vesiculosus*	4		
8229	*D. salina*	*F. vesiculosus*	4		
8230	*D. salina*	*F. vesiculosus*	4		
8231	*D. salina*	*F. vesiculosus*	4		
8232	*D. salina*	*Fucus* sp.	4		
8233	*D. salina*	*F. serratus*	4		
8520	*D. arenaria*	coastal sand	5	n. d.	Copenhagen, Denmark

Strain Nr. (NBRC)	Taxon	Substrate	Surface-sterilization [a]	Date of Isolation	Geographical Origin
8281	*D. salina*	moss	5	n. d.	McMurdo Stn. Antartic
8282	*D. salina*	n. d.	5	n. d.	n. d.
8352	*D. salina*	*Pinus* sp.	5	n. d.	United Kingdom
8353	*D. salina*	salt marsh soil	5		
32140	*D. arenaria*	driftwood	5	n. d.	Hokaido, Japan
32139	*D. salina*	seafoam	5	n. d.	Niigata, Japan
8359	*D. arenaria*	coastal sand	5	n. d.	France, Atlantic

Appendix A

Geographic Origin of the Strains of *D. arenaria* and *D. salina*

Strain Nr. (UAMH)	Taxon	Substrate	Surface-sterilization [a]	Date of Isolation	Geographical Origin
1357	*D. arenaria*	n. d.	5	n. d.	n. d.

n. d. = no data available

[a] Mode of Surface-Sterilization

1 - not surface-sterilized

2 - 70 % EtOH, 1 – 2 seconds; rinse 3x with sterile water

3 - 70 % EtOH, 30 seconds; rinse 3x with sterile water

4 - wash with sterile distilled water

5 - no information available

Appendix B

Gene Sequence Data of Marine *Dendryphiella* species

B.1. Parameters for PCR Amplification (RAPD)

	stock in M	working M	end M	µl (1)	Mix	
Sample				**5.00**	175	
Promega buffer 10-fach	10	1	1	5.00	**175**	buffer
Promega Nucleotide 10mM	1.00E-02	2.00E-03	1.60E-04	4.00	**140**	dNTPs
MgCl2 25mM	2.50E-02		3.00E-03	6.00	**210**	MgCl2
M13 100µM	1.00E-04	6.25E-06	2.00E-07	1.60	**56**	Primer
Promega Taq U/µl	5		1	0.20	**7**	Taq
Water				28.20	**987**	water
			Volume	50.00		
			Total Volume		1575	
			premix volume to pipett		**45.0**	

no. of samples + 2 = **35**

PCR

big machine	prog # **78** (M13)	
	min.	temp C
initial denaturation	1	94
40 cycles	20sec	94
	1	**50**
	30sec	72
final extension	7	72
Pause		18

Gel Electrophoresis

agarose	1.5%
bp ladder	+
dark blue dye	+
product	10µl
conditions	80 V
	200 mA
	80 min

Appendix B

B.1. Parameters for PCR Amplification (ITS 1 and 2)

	stock in M	working M	end M	µl (1)	Mix	
Sample				**5.00**	185	
Promega buffer 10 -fach	10	1	1	5.00	**185**	buffer
Promega Nucleotide 10mM	1.00E-02	2.00E-03	1.60E-04	4.00	**148**	dNTPs
MgCl2 25mM	2.50E-02		3.00E-03	6.00	**222**	MgCl2
SR6R f 100µM	1.00E-04	6.25E-06	5.00E-07	4.00	**148**	Primer
LR1 r 100µM	1.00E-04	6.25E-06	5.00E-07	4.00	**148**	Primer
Promega Taq U/µl	5		1	0.20	**7.4**	Taq
Water				21.80	**806.6**	water
			Volume	50.00		
			Total Volume		1665	
			premix volume to pipett		**45.0**	

no. of samples + 2 = **37**

PCR

big machine	prog # **5** (ITS)	
	min	temp C
initial denaturation	1	94
30 cycles	1	94
	1	50
	90 sec	72
final extension	7	72
Pause		4

Gel Electrophoresis

agarose	1.5%
bp ladder	+
dark blue dye	+
product	10µl
condition	80 V
	200 mA
	30 min

Appendix B

B.1. Parameters for PCR Amplification (*tef*1)

	stock in M	working M	end M	µl (1)	Mix	
Sample				**5.00**	190	
Promega buffer 10 -fach	10	1	1	5.00	**190**	buffer
Promega Nucleotide 10mM	1.00E-02	2.00E-03	1.60E-04	4.00	**152**	dNTPs
MgCl2 25mM	2.50E-02		3.00E-03	6.00	**228**	MgCl2
EF1-728F 100µM	1.00E-04	6.25E-06	5.00E-07	4.00	**152**	Primer
TEF-LLErev	1.00E-04	6.25E-06	5.00E-07	4.00	**152**	Primer
Promega Taq U/µl	5		1	0.20	**7.6**	Taq
Water				21.80	**828.4**	water
			Volume	50.00		
			Total Volume		1710	
			premix volume to pipett		**45.0**	
No. of samples + 2 =	38					

PCR

big machine	prog # **68** (longTEF_2004)	
	min	temp C
initial denaturation	1	94
30 cycles	1	94
	1	59
	50 sec	74
final extension	7	74
Pause		18

Gel Electrophoresis

agarose	1.5%
bp ladder	+
dark blue dye	+
product	10µl
conditions	80 V
	200 mA
	90 min

Appendix B

B.1. Parameters for PCR Amplification (*rpb2*)

	stock in M	working M	end M	µl (1)	Mix	
Sample				**5.00**	40	
Promega buffer 10 -fach	10	1	1	5.00	**40**	buffer
Promega Nucleotide 10mM	1.00E-02	2.00E-03	1.60E-04	1.00	**8**	dNTPs
MgCl2 25mM	2.50E-02		3.00E-03	5.00	**40**	MgCl2
fRPB2 5F 100µM	1.00E-04	6.25E-06	5.00E-07	2.50	**20**	Primer
fRPB2 7cR 100µM	1.00E-04	6.25E-06	5.00E-07	2.50	**20**	Primer
Promega Taq U/µl	5		1	0.50	**4**	Taq
Water				28.50	**228**	water
			Volume	50.00		
			Total Volume		360	
			premix volume to pipett		**45.0**	

No. of Samples + 2 = 8

PCR

small machine	prog #**67** (RPB2)	
	min	temp C
initial denaturation	5	95
30 cycles	1	95
	90sec	55
	90sec	72
final extension	7	72
Pause		14

Gel Electrophoresis

agarose	1.5%
bp ladder	+
dark blue dye	+
product	10µl
conditions	80 V
	200 mA
	30 min

Appendix B

B.2. Results of the BLAST nucleotide-nucleotide similarity search

1. *D. arenaria* type strains (CBS 181.58) - ITS 1 and 2 gene sequence

Sequences producing significant alignments:		Score (Bits)	E Value
gi|18139931|gb|AF442808.1|	Stemphylium sp. EGS 42-138 18S rib...	825	0.0
gi|18139923|gb|AF442800.1|	Stemphylium trifolii NO 712 18S ri...	821	0.0
gi|18139922|gb|AF442799.1|	Stemphylium trifolii NO 667 18S ri...	821	0.0
gi|18139921|gb|AF442798.1|	Stemphylium trifolii NO 553 18S ri...	821	0.0
gi|34872112|gb|AY372675.1|	Stemphylium globuliferum strain SG...	819	0.0
gi|18139928|gb|AF442805.1|	Stemphylium sp. EGS 44-149 18S rib...	817	0.0
gi|18139926|gb|AF442803.1|	Stemphylium vesicarium EGS 37-067 ...	817	0.0
gi|18139915|gb|AF442792.1|	Stemphylium majusculum EGS 29-094 ...	817	0.0
gi|18139909|gb|AF442786.1|	Pleospora herbarum EGS 30-181.1 18...	817	0.0
gi|18139907|gb|AF442784.1|	Stemphylium gracilariae EGS 37-073...	817	0.0
gi|18139901|gb|AF442778.1|	Stemphylium astragali EGS 29-062 1...	817	0.0
gi|18139932|gb|AF442809.1|	Stemphylium sp. EGS 48-077 18S rib...	809	0.0
gi|18139908|gb|AF442785.1|	Pleospora herbarum EGS 36-138.2 ex...	809	0.0
gi|18139910|gb|AF442787.1|	Stemphylium lancipes EGS 46-182 18...	801	0.0
gi|18139902|gb|AF442779.1|	Stemphylium astragali EGS 27-194.2...	801	0.0
gi|37551766|gb|AY329270.1|	Pleospora sp. P343 internal transc...	791	0.0
gi|37551765|gb|AY329269.1|	Pleospora sp. P342 internal transc...	791	0.0
gi|37551697|gb|AY329201.1|	Stemphylium sp. EGS42-138 internal...	791	0.0
gi|37551666|gb|AY329170.1|	Pleospora sp. P56 internal transcr...	791	0.0
gi|37551714|gb|AY329218.1|	Stemphylium trifolii strain EGS12-...	787	0.0
gi|37551762|gb|AY329266.1|	Stemphylium sp. EGS49-054 internal...	783	0.0
gi|37551761|gb|AY329265.1|	Stemphylium sp. EGS49-053 internal...	783	0.0
gi|37551760|gb|AY329264.1|	Stemphylium sp. EGS49-052 internal...	783	0.0
gi|37551759|gb|AY329263.1|	Stemphylium sp. EGS49-051 internal...	783	0.0
gi|37551758|gb|AY329262.1|	Stemphylium sp. EGS49-050 internal...	783	0.0
gi|37551755|gb|AY329259.1|	Stemphylium sp. EGS49-046 internal...	783	0.0
gi|37551754|gb|AY329258.1|	Stemphylium sp. EGS49-045 internal...	783	0.0
gi|37551751|gb|AY329255.1|	Stemphylium sp. EGS49-042 internal...	783	0.0
gi|37551750|gb|AY329254.1|	Stemphylium sp. EGS49-041 internal...	783	0.0
gi|37551747|gb|AY329251.1|	Stemphylium sp. EGS49-038 internal...	783	0.0
gi|37551744|gb|AY329248.1|	Stemphylium sp. EGS49-035 internal...	783	0.0
gi|37551741|gb|AY329245.1|	Stemphylium sp. EGS49-030 internal...	783	0.0
gi|37551739|gb|AY329243.1|	Stemphylium sp. P301 internal tran...	783	0.0
gi|37551738|gb|AY329242.1|	Stemphylium sp. EGS48-175 internal...	783	0.0
gi|37551737|gb|AY329241.1|	Stemphylium sp. EGS48-173 internal...	783	0.0
gi|37551736|gb|AY329240.1|	Stemphylium sp. EGS48-172 internal...	783	0.0
gi|37551735|gb|AY329239.1|	Stemphylium sp. EGS48-171 internal...	783	0.0
gi|37551734|gb|AY329238.1|	Stemphylium sp. EGS48-170 internal...	783	0.0
gi|37551733|gb|AY329237.1|	Stemphylium sp. EGS48-169 internal...	783	0.0
gi|37551732|gb|AY329236.1|	Stemphylium sp. EGS48-168 internal...	783	0.0
gi|37551731|gb|AY329235.1|	Stemphylium sp. EGS48-167 internal...	783	0.0
gi|37551730|gb|AY329234.1|	Stemphylium sp. EGS48-165 internal...	783	0.0
gi|37551729|gb|AY329233.1|	Stemphylium sp. EGS48-163 internal...	783	0.0
gi|37551728|gb|AY329232.1|	Pleospora sedicola strain EGS48-09...	783	0.0
gi|37551725|gb|AY329229.1|	Pleospora tomatonis strain EGS29-0...	783	0.0
gi|37551724|gb|AY329228.1|	Stemphylium majusculum strain EGS1...	783	0.0
gi|37551713|gb|AY329217.1|	Pleospora gracilariae strain EGS37...	783	0.0
gi|37551708|gb|AY329212.1|	Pleospora sp. EGS37-067 internal t...	783	0.0
gi|37551707|gb|AY329211.1|	Stemphylium sp. EGS35-187 internal...	783	0.0
gi|37551706|gb|AY329210.1|	Stemphylium sp. EGS35-171 internal...	783	0.0
gi|37551705|gb|AY329209.1|	Stemphylium sp. EGS35-170 internal...	783	0.0

Appendix B

B.2. Results of the BLAST nucleotide-nucleotide similarity search

1. *D. arenaria* type strains (CBS 181.58) - ITS 1 and 2 gene sequence, cont.

Sequences producing significant alignments:		Score (Bits)	E Value
gi|37551704|gb|AY329208.1|	Stemphylium sp. EGS35-169 internal...	783	0.0
gi|37551703|gb|AY329207.1|	Stemphylium sp. EGS35-163 internal...	783	0.0
gi|37551701|gb|AY329205.1|	Stemphylium sp. EGS47-135 internal...	783	0.0
gi|37551700|gb|AY329204.1|	Stemphylium sp. EGS47-132 internal...	783	0.0
gi|37551698|gb|AY329202.1|	Stemphylium sp. EGS44-149 internal...	783	0.0
gi|37551696|gb|AY329200.1|	Stemphylium sp. EGS42-022 internal...	783	0.0
gi|37551694|gb|AY329198.1|	Stemphylium sp. EGS40-038 internal...	783	0.0
gi|37551693|gb|AY329197.1|	Stemphylium sp. EGS38-091 internal...	783	0.0
gi|37551691|gb|AY329195.1|	Stemphylium sp. EGS38-089 internal...	783	0.0
gi|37551690|gb|AY329194.1|	Stemphylium sp. EGS35-190 internal...	783	0.0
gi|37551689|gb|AY329193.1|	Stemphylium sp. EGS48-105 internal...	783	0.0
gi|37551688|gb|AY329192.1|	Stemphylium sp. EGS48-104 internal...	783	0.0
gi|37551686|gb|AY329190.1|	Stemphylium sp. EGS48-102 internal...	783	0.0
gi|37551684|gb|AY329188.1|	Stemphylium sp. EGS48-099 internal...	783	0.0
gi|37551681|gb|AY329185.1|	Stemphylium sp. EGS48-087 internal...	783	0.0
gi|37551680|gb|AY329184.1|	Stemphylium sp. EGS48-075 internal...	783	0.0
gi|37551678|gb|AY329182.1|	Stemphylium sp. EGS30-181 internal...	783	0.0
gi|37551677|gb|AY329181.1|	Stemphylium sp. EGS29-062 internal...	783	0.0
gi|37551676|gb|AY329180.1|	Stemphylium sp. EGS27-1942 interna...	783	0.0
gi|37551675|gb|AY329179.1|	Stemphylium sp. EGS27-1941 interna...	783	0.0
gi|37551674|gb|AY329178.1|	Stemphylium sp. EGS08-174 internal...	783	0.0
gi|37551673|gb|AY329177.1|	Pleospora gigaspora strain EGS37-0...	783	0.0
gi|37551672|gb|AY329176.1|	Pleospora sp. EGS37-149 internal t...	783	0.0
gi|37551670|gb|AY329174.1|	Pleospora gigaspora strain EGS37-0...	783	0.0
gi|37551668|gb|AY329172.1|	Pleospora sp. P93 internal transcr...	783	0.0
gi|37551667|gb|AY329171.1|	Pleospora alfalfae strain EGS36-08...	783	0.0
gi|37551665|gb|AY329169.1|	Pleospora herbarum strain EGS36-13...	783	0.0
gi|37551716|gb|AY329220.1|	Stemphylium sp. EGS31-008 internal...	781	0.0
gi|45736341|dbj|AB120853.1|	Embellisia annulata genes for ITS...	779	0.0
gi|37551757|gb|AY329261.1|	Stemphylium sp. EGS49-048 internal...	775	0.0
gi|37551756|gb|AY329260.1|	Stemphylium sp. EGS49-047 internal...	775	0.0
gi|37551753|gb|AY329257.1|	Stemphylium sp. EGS49-044 internal...	775	0.0
gi|37551748|gb|AY329252.1|	Stemphylium sp. EGS49-039 internal...	775	0.0
gi|37551740|gb|AY329244.1|	Stemphylium sp. EGS49-029 internal...	775	0.0
gi|37551726|gb|AY329230.1|	Pleospora eturmiuna strain EGS29-0...	775	0.0
gi|37551695|gb|AY329199.1|	Stemphylium sp. EGS41-194 internal...	775	0.0
gi|37551685|gb|AY329189.1|	Stemphylium sp. EGS48-101 internal...	775	0.0
gi|37551679|gb|AY329183.1|	Stemphylium sp. EGS48-074 internal...	775	0.0
gi|7546968|gb|AF229483.1|AF229483	Stemphylium sarcinaeforme s...	775	0.0
gi|18139916|gb|AF442793.1|	Stemphylium sarciniforme EGS 38-12...	771	0.0
gi|37551699|gb|AY329203.1|	Stemphylium lancipes strain EGS46-...	767	0.0
gi|37551727|gb|AY329231.1|	Pleospora paludiscirpi strain EGS3...	761	0.0
gi|18139924|gb|AF442801.1|	Stemphylium trifolii NO 615 18S ri...	759	0.0
gi|84782464|gb|DQ323706.1|	Pleospora eturmiuna isolate Riv-St...	753	0.0
gi|37551746|gb|AY329250.1|	Stemphylium sp. EGS49-037 internal...	737	0.0
gi|37551745|gb|AY329249.1|	Stemphylium sp. EGS49-036 internal...	737	0.0
gi|37551742|gb|AY329246.1|	Stemphylium sp. EGS49-033 internal...	737	0.0
gi|37551717|gb|AY329221.1|	Stemphylium sarciniforme strain EG...	737	0.0
gi|37551709|gb|AY329213.1|	Stemphylium sarciniforme strain EG...	737	0.0

Appendix B

B.2. Results of the BLAST nucleotide-nucleotide similarity search

1. *D. salina* reference strain (CBS 142.50) - ITS 1 and 2 gene sequence

Sequences producing significant alignments:	Score (Bits)	E Value
gi|6625901|gb|AF203450.1| Stemphylium solani strain SS31 18S ...	948	0.0
gi|6625900|gb|AF203449.1| Stemphylium solani strain SS28 18S ...	948	0.0
gi|6625899|gb|AF203448.1| Stemphylium solani strain SS21 18S ...	948	0.0
gi|451838|gb|U05202.1|PHU05202 Pleospora herbarum DAOM 150679...	928	0.0
gi|6009744|dbj|AB026165.1| Pleospora herbarum gene for 18S rR...	922	0.0
gi|6009743|dbj|AB026164.1| Pleospora herbarum gene for 18S rR...	922	0.0
gi|6009742|dbj|AB026163.1| Pleospora herbarum gene for 18S rR...	922	0.0
gi|7546969|gb|AF229484.1|AF229484 Stemphylium vesicarium stra...	920	0.0
gi|7546964|gb|AF229479.1|AF229479 Pleospora herbarum strain A...	920	0.0
gi|3688538|emb|Y17068.1|SBY17068 Stemphylium botryosum rRNA g...	912	0.0
gi|6625902|gb|AF203451.1| Stemphylium solani strain SS1 18S r...	908	0.0
gi|34872112|gb|AY372675.1| Stemphylium globuliferum strain SG...	888	0.0
gi|7546966|gb|AF229481.1|AF229481 Pleospora tarda strain ATCC...	878	0.0
gi|5031118|gb|AF071343.1|AF071343 Stemphylium alfalfae 18S ri...	874	0.0
gi|34872134|gb|AY372679.1| Stemphylium nabarii strain SN22 18...	870	0.0
gi|45736341|dbj|AB120853.1| Embellisia annulata genes for ITS...	864	0.0
gi|5031120|gb|AF071345.1|AF071345 Pleospora herbarum 18S ribo...	856	0.0
gi|18139915|gb|AF442792.1| Stemphylium majusculum EGS 29-094 ...	852	0.0
gi|18139928|gb|AF442805.1| Stemphylium sp. EGS 44-149 18S rib...	846	0.0
gi|18139926|gb|AF442803.1| Stemphylium vesicarium EGS 37-067 ...	846	0.0
gi|18139909|gb|AF442786.1| Pleospora herbarum EGS 30-181.1 18...	846	0.0
gi|18139899|gb|AF442776.1| Stemphylium alfalfae EGS 40-038 18...	846	0.0
gi|18139897|gb|AF442774.1| Stemphylium alfalfae EGS 39-127 18...	846	0.0
gi|84796520|gb|DQ335976.1| Stemphylium sp. SC28 internal tran...	844	0.0
gi|54111960|gb|AY751454.1| Stemphylium subglobuliferum strain...	842	0.0
gi|18139908|gb|AF442785.1| Pleospora herbarum EGS 36-138.2 ex...	839	0.0
gi|18139932|gb|AF442809.1| Stemphylium sp. EGS 48-077 18S rib...	837	0.0
gi|18139931|gb|AF442808.1| Stemphylium sp. EGS 42-138 18S rib...	837	0.0
gi|18139910|gb|AF442787.1| Stemphylium lancipes EGS 46-182 18...	837	0.0
gi|16194986|gb|AF426739.1|AF426739 Stemphylium solani 18S rib...	835	0.0
gi|18139923|gb|AF442800.1| Stemphylium trifolii NO 712 18S ri...	829	0.0
gi|18139922|gb|AF442799.1| Stemphylium trifolii NO 667 18S ri...	829	0.0
gi|18139921|gb|AF442798.1| Stemphylium trifolii NO 553 18S ri...	829	0.0
gi|84782464|gb|DQ323706.1| Pleospora eturmiuna isolate Riv-St...	825	0.0
gi|7546967|gb|AF229482.1|AF229482 Stemphylium callistephi str...	823	0.0
gi|7546968|gb|AF229483.1|AF229483 Stemphylium sarcinaeforme s...	815	0.0
gi|37551762|gb|AY329266.1| Stemphylium sp. EGS49-054 internal...	811	0.0
gi|37551761|gb|AY329265.1| Stemphylium sp. EGS49-053 internal...	811	0.0
gi|37551760|gb|AY329264.1| Stemphylium sp. EGS49-052 internal...	811	0.0
gi|37551759|gb|AY329263.1| Stemphylium sp. EGS49-051 internal...	811	0.0
gi|37551758|gb|AY329262.1| Stemphylium sp. EGS49-050 internal...	811	0.0
gi|37551754|gb|AY329258.1| Stemphylium sp. EGS49-045 internal...	811	0.0
gi|37551750|gb|AY329254.1| Stemphylium sp. EGS49-041 internal...	811	0.0
gi|37551744|gb|AY329248.1| Stemphylium sp. EGS49-035 internal...	811	0.0
gi|37551739|gb|AY329243.1| Stemphylium sp. P301 internal tran...	811	0.0
gi|37551738|gb|AY329242.1| Stemphylium sp. EGS48-175 internal...	811	0.0
gi|37551737|gb|AY329241.1| Stemphylium sp. EGS48-173 internal...	811	0.0
gi|37551736|gb|AY329240.1| Stemphylium sp. EGS48-172 internal...	811	0.0
gi|37551735|gb|AY329239.1| Stemphylium sp. EGS48-171 internal...	811	0.0
gi|37551734|gb|AY329238.1| Stemphylium sp. EGS48-170 internal...	811	0.0
gi|37551733|gb|AY329237.1| Stemphylium sp. EGS48-169 internal...	811	0.0

Appendix B

B.2. Results of the BLAST nucleotide-nucleotide similarity search

1. *D. salina* reference strain (CBS 142.50) - ITS 1 and 2 gene sequence, cont.

Sequences producing significant alignments:		Score (Bits)	E Value
gi|37551732|gb|AY329236.1|	Stemphylium sp. EGS48-168 internal...	811	0.0
gi|37551731|gb|AY329235.1|	Stemphylium sp. EGS48-167 internal...	811	0.0
gi|37551730|gb|AY329234.1|	Stemphylium sp. EGS48-165 internal...	811	0.0
gi|37551729|gb|AY329233.1|	Stemphylium sp. EGS48-163 internal...	811	0.0
gi|37551728|gb|AY329232.1|	Pleospora sedicola strain EGS48-09...	811	0.0
gi|37551725|gb|AY329229.1|	Pleospora tomatonis strain EGS29-0...	811	0.0
gi|37551708|gb|AY329212.1|	Pleospora sp. EGS37-067 internal t...	811	0.0
gi|37551707|gb|AY329211.1|	Stemphylium sp. EGS35-187 internal...	811	0.0
gi|37551706|gb|AY329210.1|	Stemphylium sp. EGS35-171 internal...	811	0.0
gi|37551705|gb|AY329209.1|	Stemphylium sp. EGS35-170 internal...	811	0.0
gi|37551704|gb|AY329208.1|	Stemphylium sp. EGS35-169 internal...	811	0.0
gi|37551703|gb|AY329207.1|	Stemphylium sp. EGS35-163 internal...	811	0.0
gi|37551701|gb|AY329205.1|	Stemphylium sp. EGS47-135 internal...	811	0.0
gi|37551700|gb|AY329204.1|	Stemphylium sp. EGS47-132 internal...	811	0.0
gi|37551698|gb|AY329202.1|	Stemphylium sp. EGS44-149 internal...	811	0.0
gi|37551696|gb|AY329200.1|	Stemphylium sp. EGS42-022 internal...	811	0.0
gi|37551694|gb|AY329198.1|	Stemphylium sp. EGS40-038 internal...	811	0.0
gi|37551693|gb|AY329197.1|	Stemphylium sp. EGS38-091 internal...	811	0.0
gi|37551691|gb|AY329195.1|	Stemphylium sp. EGS38-089 internal...	811	0.0
gi|37551690|gb|AY329194.1|	Stemphylium sp. EGS35-190 internal...	811	0.0
gi|37551689|gb|AY329193.1|	Stemphylium sp. EGS48-105 internal...	811	0.0
gi|37551688|gb|AY329192.1|	Stemphylium sp. EGS48-104 internal...	811	0.0
gi|37551686|gb|AY329190.1|	Stemphylium sp. EGS48-102 internal...	811	0.0
gi|37551684|gb|AY329188.1|	Stemphylium sp. EGS48-099 internal...	811	0.0
gi|37551681|gb|AY329185.1|	Stemphylium sp. EGS48-087 internal...	811	0.0
gi|37551680|gb|AY329184.1|	Stemphylium sp. EGS48-075 internal...	811	0.0
gi|37551678|gb|AY329182.1|	Stemphylium sp. EGS30-181 internal...	811	0.0
gi|37551672|gb|AY329176.1|	Pleospora sp. EGS37-149 internal t...	811	0.0
gi|37551668|gb|AY329172.1|	Pleospora sp. P93 internal transcr...	811	0.0
gi|37551667|gb|AY329171.1|	Pleospora alfalfae strain EGS36-08...	811	0.0
gi|37551665|gb|AY329169.1|	Pleospora herbarum strain EGS36-13...	811	0.0
gi|37551766|gb|AY329270.1|	Pleospora sp. P343 internal transc...	801	0.0
gi|37551765|gb|AY329269.1|	Pleospora sp. P342 internal transc...	801	0.0
gi|37551757|gb|AY329261.1|	Stemphylium sp. EGS49-048 internal...	801	0.0
gi|37551756|gb|AY329260.1|	Stemphylium sp. EGS49-047 internal...	801	0.0
gi|37551699|gb|AY329203.1|	Stemphylium lancipes strain EGS46-...	801	0.0
gi|37551697|gb|AY329201.1|	Stemphylium sp. EGS42-138 internal...	801	0.0
gi|37551666|gb|AY329170.1|	Pleospora sp. P56 internal transcr...	801	0.0
gi|37551714|gb|AY329218.1|	Stemphylium trifolii strain EGS12-...	795	0.0
gi|37551716|gb|AY329220.1|	Stemphylium sp. EGS31-008 internal...	791	0.0
gi|37551727|gb|AY329231.1|	Pleospora paludiscirpi strain EGS3...	787	0.0
gi|18139916|gb|AF442793.1|	Stemphylium sarciniforme EGS 38-12...	781	0.0
gi|18139924|gb|AF442801.1|	Stemphylium trifolii NO 615 18S ri...	767	0.0
gi|45736353|dbj|AB120865.1|	Pleospora tarda genes for ITS1, 5...	755	0.0
gi|37551746|gb|AY329250.1|	Stemphylium sp. EGS49-037 internal...	747	0.0
gi|37551745|gb|AY329249.1|	Stemphylium sp. EGS49-036 internal...	747	0.0
gi|37551742|gb|AY329246.1|	Stemphylium sp. EGS49-033 internal...	747	0.0
gi|37551717|gb|AY329221.1|	Stemphylium sarciniforme strain EG...	747	0.0
gi|37551709|gb|AY329213.1|	Stemphylium sarciniforme strain EG...	747	0.0

Appendix B

B.2. Results of the BLAST nucleotide-nucleotide similarity search

2. *D. arenaria* type strains (CBS 181.58) - *tef*1 gene sequence

Sequences producing significant alignments:		Score (Bits)	E Value
gi|37519148|gb|AY324765.1|	Stemphylium sp. EGS45-031 elongati...	1023	0.0
gi|37519146|gb|AY324764.1|	Stemphylium sp. EGS47-197 elongati...	1023	0.0
gi|37519134|gb|AY324758.1|	Stemphylium xanthosomatis EGS17-13...	1023	0.0
gi|46909173|gb|AY534633.1|	Stemphylium sp. SS1 elongation fac...	1023	0.0
gi|37519158|gb|AY324770.1|	Stemphylium sp. EGS49-043 elongati...	1019	0.0
gi|37519150|gb|AY324766.1|	Stemphylium sp. EGS45-036 elongati...	1019	0.0
gi|37519144|gb|AY324763.1|	Stemphylium sp. EGS46-183 elongati...	1019	0.0
gi|37519142|gb|AY324762.1|	Stemphylium sp. EGS29-161 elongati...	1019	0.0
gi|37519140|gb|AY324761.1|	Stemphylium lycopersici EGS46-001 ...	1019	0.0
gi|37519162|gb|AY324772.1|	Pleospora sp. P338 elongation factor-	989	0.0
gi|37519160|gb|AY324771.1|	Pleospora sp. P327 elongation factor-	989	0.0
gi|37519128|gb|AY324755.1|	Stemphylium sp. EGS48-097 elongati...	987	0.0
gi|37519122|gb|AY324752.1|	Pleospora sp. P107 elongation factor-	987	0.0
gi|37519126|gb|AY324754.1|	Stemphylium sp. EGS48-089 elongati...	985	0.0
gi|37519130|gb|AY324756.1|	Stemphylium sp. EGS38-090 elongati...	979	0.0
gi|37519166|gb|AY324774.1|	Pleospora sp. P343 elongation factor-	971	0.0
gi|37519164|gb|AY324773.1|	Pleospora sp. P342 elongation factor-	971	0.0
gi|37519132|gb|AY324757.1|	Stemphylium sp. EGS42-138 elongati...	971	0.0
gi|37519120|gb|AY324751.1|	Pleospora sp. P56 elongation factor-1	963	0.0
gi|37519156|gb|AY324769.1|	Pleospora paludiscirpi EGS31-016 e...	942	0.0
gi|37519170|gb|AY324776.1|	Stemphylium loti NO 1364 elongatio...	870	0.0
gi|37519168|gb|AY324775.1|	Stemphylium loti NO 0770 elongatio...	870	0.0
gi|32993518|dbj|AK108309.1|	Oryza sativa (japonica cultivar-g...	656	0.0
gi|643454|gb|U19723.1|APU19723	Aureobasidium pullulans transl...	636	3e-179
gi|37519124|gb|AY324753.1|	Pleospora triglochinicola EGS36-11...	618	6e-174
gi|32992575|dbj|AK107366.1|	Oryza sativa (japonica cultivar-g...	618	6e-174
gi|53831017|gb|AY531967.1|	Cordyceps bassiana isolate Sc2533 ...	607	2e-170
gi|38324515|gb|AY445082.1|	Metarhizium anisopliae strain E6 t...	593	4e-166
gi|53830921|gb|AY531917.1|	Cordyceps bassiana isolate 2641 tr...	593	4e-166
gi|53830901|gb|AY531907.1|	Cordyceps bassiana isolate 1969 tr...	593	4e-166
gi|37518966|gb|AY324674.1|	Stemphylium sp. EGS49-040 elongati...	589	6e-165
gi|37518964|gb|AY324673.1|	Stemphylium sp. EGS49-034 elongati...	589	6e-165
gi|37518962|gb|AY324672.1|	Stemphylium sp. EGS48-103 elongati...	589	6e-165
gi|37518960|gb|AY324671.1|	Pleospora tarda elongation factor-1 a	589	6e-165
gi|37519118|gb|AY324750.1|	Stemphylium callistephi NO 0536 el...	587	2e-164
gi|37518978|gb|AY324680.1|	Pleospora gigaspora EGS37-017 elon...	587	2e-164
gi|37518974|gb|AY324678.1|	Pleospora gigaspora EGS37-016 elon...	587	2e-164
gi|37519138|gb|AY324760.1|	Stemphylium solani EGS42-027 elong...	585	9e-164
gi|53830945|gb|AY531929.1|	Cordyceps bassiana isolate 326 tra...	585	9e-164
gi|53830935|gb|AY531924.1|	Cordyceps bassiana isolate 300 tra...	585	9e-164
gi|53830917|gb|AY531915.1|	Cordyceps bassiana isolate 2567 tr...	585	9e-164
gi|53830911|gb|AY531912.1|	Cordyceps bassiana isolate 2251 tr...	585	9e-164
gi|37519154|gb|AY324768.1|	Stemphylium sp. EGS44-070 elongati...	583	4e-163
gi|37519152|gb|AY324767.1|	Stemphylium sp. EGS42-055 elongati...	583	4e-163
gi|37519136|gb|AY324759.1|	Stemphylium solani EGS41-135 elong...	583	4e-163
gi|46909179|gb|AY534636.1|	Stemphylium sp. SS31 elongation fa...	583	4e-163
gi|46909177|gb|AY534635.1|	Stemphylium sp. SS28 elongation fa...	583	4e-163
gi|46909175|gb|AY534634.1|	Stemphylium sp. SS21 elongation fa...	583	4e-163
gi|37519102|gb|AY324742.1|	Stemphylium lancipes EGS46-182 elo...	581	1e-162
gi|37519090|gb|AY324736.1|	Stemphylium sp. EGS49-048 elongati...	581	1e-162
gi|37519088|gb|AY324735.1|	Stemphylium sp. EGS49-047 elongati...	581	1e-162

Appendix B

B.2. Results of the BLAST nucleotide-nucleotide similarity search

2. *D. arenaria* type strains (CBS 181.58) - *tef*1 gene sequence, cont.

Sequences producing significant alignments:		Score (Bits)	E Value
gi|37519082|gb|AY324732.1|	Stemphylium sp. EGS49-044 elongati...	581	1e-162
gi|37519076|gb|AY324729.1|	Stemphylium sp. EGS49-039 elongati...	581	1e-162
gi|37519068|gb|AY324725.1|	Stemphylium sp. EGS49-029 elongati...	581	1e-162
gi|37519042|gb|AY324712.1|	Pleospora eturmiuna EGS29-099 elon...	581	1e-162
gi|37519014|gb|AY324698.1|	Stemphylium sp. EGS41-194 elongati...	581	1e-162
gi|37518998|gb|AY324690.1|	Stemphylium sp. EGS48-101 elongati...	581	1e-162
gi|37518990|gb|AY324686.1|	Stemphylium sp. EGS48-074 elongati...	581	1e-162
gi|37519116|gb|AY324749.1|	Stemphylium sp. EGS49-037 elongati...	579	6e-162
gi|37519114|gb|AY324748.1|	Stemphylium sp. EGS49-036 elongati...	579	6e-162
gi|37519112|gb|AY324747.1|	Stemphylium sp. EGS49-033 elongati...	579	6e-162
gi|37519110|gb|AY324746.1|	Stemphylium sarciniforme EGS29-188...	579	6e-162
gi|37519108|gb|AY324745.1|	Stemphylium sp. EGS31-008 elongati...	579	6e-162
gi|37519106|gb|AY324744.1|	Stemphylium trifolii EGS12-142 elo...	579	6e-162
gi|37519104|gb|AY324743.1|	Stemphylium sarciniforme EGS38-121...	579	6e-162
gi|87132975|gb|DQ376246.1|	Beauveria sp. IMI 228343 elongatio...	577	2e-161
gi|53830915|gb|AY531914.1|	Cordyceps bassiana isolate 2544 tr...	575	9e-161
gi|37519074|gb|AY324728.1|	Stemphylium sp. EGS49-038 elongati...	573	3e-160
gi|37519038|gb|AY324710.1|	Stemphylium majusculum EGS16-068 e...	573	3e-160
gi|37518986|gb|AY324684.1|	Stemphylium sp. EGS29-062 elongati...	573	3e-160
gi|37518984|gb|AY324683.1|	Stemphylium sp. EGS27-1942 elongat...	573	3e-160
gi|37518982|gb|AY324682.1|	Stemphylium sp. EGS27-1941 elongat...	573	3e-160
gi|37518980|gb|AY324681.1|	Stemphylium sp. EGS08-174 elongati...	573	3e-160
gi|62548282|gb|AY883712.1|	Cordyceps bassiana strain ARSEF 72...	569	5e-159
gi|62548280|gb|AY883711.1|	Cordyceps bassiana strain ARSEF 72...	569	5e-159
gi|62548276|gb|AY883709.1|	Cordyceps bassiana strain ARSEF 72...	569	5e-159
gi|62548272|gb|AY883707.1|	Cordyceps bassiana strain ARSEF 72...	569	5e-159
gi|62548266|gb|AY883704.1|	Cordyceps bassiana strain ARSEF 72...	569	5e-159
gi|62548262|gb|AY883702.1|	Cordyceps bassiana strain ARSEF 72...	569	5e-159
gi|62548254|gb|AY883698.1|	Cordyceps bassiana strain ARSEF 43...	569	5e-159
gi|62548252|gb|AY883697.1|	Cordyceps bassiana strain ARSEF 24...	569	5e-159
gi|62548250|gb|AY883696.1|	Cordyceps bassiana strain ARSEF 16...	569	5e-159
gi|62548238|gb|AY883690.1|	Cordyceps bassiana strain ARSEF 10...	569	5e-159
gi|53831015|gb|AY531966.1|	Cordyceps bassiana isolate Cs252 t...	569	5e-159
gi|53831007|gb|AY531962.1|	Cordyceps bassiana isolate 816 tra...	569	5e-159
gi|53830986|gb|AY531951.1|	Cordyceps bassiana isolate 714 tra...	569	5e-159
gi|53851041|gb|AY531949.1|	Cordyceps bassiana isolate 7044 tr...	569	5e-159
gi|53851039|gb|AY531948.1|	Cordyceps bassiana isolate 7043 tr...	569	5e-159
gi|53830965|gb|AY531939.1|	Cordyceps bassiana isolate 5689 tr...	569	5e-159
gi|53830953|gb|AY531933.1|	Cordyceps bassiana isolate 4021 tr...	569	5e-159
gi|53830927|gb|AY531920.1|	Cordyceps bassiana isolate 2922 tr...	569	5e-159
gi|53830905|gb|AY531909.1|	Cordyceps bassiana isolate 1988 tr...	569	5e-159
gi|53830895|gb|AY531904.1|	Cordyceps bassiana isolate 1848 tr...	569	5e-159
gi|53830887|gb|AY531900.1|	Cordyceps bassiana isolate 1802 tr...	569	5e-159
gi|53830885|gb|AY531899.1|	Cordyceps bassiana isolate 1685 tr...	569	5e-159
gi|53830879|gb|AY531896.1|	Cordyceps bassiana isolate 1628 tr...	569	5e-159
gi|53830863|gb|AY531888.1|	Cordyceps bassiana isolate 1398 tr...	569	5e-159
gi|53830859|gb|AY531886.1|	Cordyceps bassiana isolate 1185 tr...	569	5e-159
gi|53830855|gb|AY531884.1|	Cordyceps bassiana isolate 1153 tr...	569	5e-159
gi|37519100|gb|AY324741.1|	Stemphylium sp. EGS49-054 elongati...	565	8e-158

Appendix B

B.2. Results of the BLAST nucleotide-nucleotide similarity search

2. *D. salina* reference strain (CBS 142.50) - *tef*1 gene sequence

Sequences producing significant alignments:		Score (Bits)	E Value
gi|37519148|gb|AY324765.1|	Stemphylium sp. EGS45-031 elongati...	1049	0.0
gi|37519146|gb|AY324764.1|	Stemphylium sp. EGS47-197 elongati...	1049	0.0
gi|37519134|gb|AY324758.1|	Stemphylium xanthosomatis EGS17-13...	1049	0.0
gi|46909173|gb|AY534633.1|	Stemphylium sp. SS1 elongation fac...	1049	0.0
gi|37519158|gb|AY324770.1|	Stemphylium sp. EGS49-043 elongati...	1041	0.0
gi|37519150|gb|AY324766.1|	Stemphylium sp. EGS45-036 elongati...	1041	0.0
gi|37519144|gb|AY324763.1|	Stemphylium sp. EGS46-183 elongati...	1041	0.0
gi|37519142|gb|AY324762.1|	Stemphylium sp. EGS29-161 elongati...	1041	0.0
gi|37519140|gb|AY324761.1|	Stemphylium lycopersici EGS46-001 ...	1041	0.0
gi|37519162|gb|AY324772.1|	Pleospora sp. P338 elongation factor-	1015	0.0
gi|37519160|gb|AY324771.1|	Pleospora sp. P327 elongation factor-	1015	0.0
gi|37519126|gb|AY324754.1|	Stemphylium sp. EGS48-089 elongati...	997	0.0
gi|37519128|gb|AY324755.1|	Stemphylium sp. EGS48-097 elongati...	993	0.0
gi|37519122|gb|AY324752.1|	Pleospora sp. P107 elongation factor-	993	0.0
gi|37519130|gb|AY324756.1|	Stemphylium sp. EGS38-090 elongati...	985	0.0
gi|37519166|gb|AY324774.1|	Pleospora sp. P343 elongation factor-	977	0.0
gi|37519164|gb|AY324773.1|	Pleospora sp. P342 elongation factor-	977	0.0
gi|37519132|gb|AY324757.1|	Stemphylium sp. EGS42-138 elongati...	977	0.0
gi|37519120|gb|AY324751.1|	Pleospora sp. P56 elongation factor-1	969	0.0
gi|37519124|gb|AY324753.1|	Pleospora triglochinicola EGS36-11...	952	0.0
gi|37519156|gb|AY324769.1|	Pleospora paludiscirpi EGS31-016 e...	950	0.0
gi|37519170|gb|AY324776.1|	Stemphylium loti NO 1364 elongatio...	860	0.0
gi|37519168|gb|AY324775.1|	Stemphylium loti NO 0770 elongatio...	860	0.0
gi|32993518|dbj|AK108309.1|	Oryza sativa (japonica cultivar-g...	605	9e-170
gi|37518966|gb|AY324674.1|	Stemphylium sp. EGS49-040 elongati...	597	2e-167
gi|37518964|gb|AY324673.1|	Stemphylium sp. EGS49-034 elongati...	597	2e-167
gi|37518962|gb|AY324672.1|	Stemphylium sp. EGS48-103 elongati...	597	2e-167
gi|37518960|gb|AY324671.1|	Pleospora tarda elongation factor-1 a	597	2e-167
gi|37519118|gb|AY324750.1|	Stemphylium callistephi NO 0536 el...	595	9e-167
gi|37518978|gb|AY324680.1|	Pleospora gigaspora EGS37-017 elon...	595	9e-167
gi|37518974|gb|AY324678.1|	Pleospora gigaspora EGS37-016 elon...	595	9e-167
gi|32992575|dbj|AK107366.1|	Oryza sativa (japonica cultivar-g...	595	9e-167
gi|37519138|gb|AY324760.1|	Stemphylium solani EGS42-027 elong...	593	4e-166
gi|37519154|gb|AY324768.1|	Stemphylium sp. EGS44-070 elongati...	591	1e-165
gi|37519152|gb|AY324767.1|	Stemphylium sp. EGS42-055 elongati...	591	1e-165
gi|37519136|gb|AY324759.1|	Stemphylium solani EGS41-135 elong...	591	1e-165
gi|46909179|gb|AY534636.1|	Stemphylium sp. SS31 elongation fa...	591	1e-165
gi|46909177|gb|AY534635.1|	Stemphylium sp. SS28 elongation fa...	591	1e-165
gi|46909175|gb|AY534634.1|	Stemphylium sp. SS21 elongation fa...	591	1e-165
gi|37519102|gb|AY324742.1|	Stemphylium lancipes EGS46-182 elo...	589	6e-165
gi|37519090|gb|AY324736.1|	Stemphylium sp. EGS49-048 elongati...	589	6e-165
gi|37519088|gb|AY324735.1|	Stemphylium sp. EGS49-047 elongati...	589	6e-165
gi|37519082|gb|AY324732.1|	Stemphylium sp. EGS49-044 elongati...	589	6e-165
gi|37519076|gb|AY324729.1|	Stemphylium sp. EGS49-039 elongati...	589	6e-165
gi|37519068|gb|AY324725.1|	Stemphylium sp. EGS49-029 elongati...	589	6e-165
gi|37519042|gb|AY324712.1|	Pleospora eturmiuna EGS29-099 elon...	589	6e-165
gi|37519014|gb|AY324698.1|	Stemphylium sp. EGS41-194 elongati...	589	6e-165
gi|37518998|gb|AY324690.1|	Stemphylium sp. EGS48-101 elongati...	589	6e-165
gi|37518990|gb|AY324686.1|	Stemphylium sp. EGS48-074 elongati...	589	6e-165
gi|37519116|gb|AY324749.1|	Stemphylium sp. EGS49-037 elongati...	587	2e-164
gi|37519114|gb|AY324748.1|	Stemphylium sp. EGS49-036 elongati...	587	2e-164

Appendix B

B.2. Results of the BLAST nucleotide-nucleotide similarity search

2. *D. salina* reference strain (CBS 142.50) - *tef*1 gene sequence, cont.

Sequences producing significant alignments:		Score (Bits)	E Value
gi|37519112|gb|AY324747.1|	Stemphylium sp. EGS49-033 elongati...	587	2e-164
gi|37519110|gb|AY324746.1|	Stemphylium sarciniforme EGS29-188...	587	2e-164
gi|37519108|gb|AY324745.1|	Stemphylium sp. EGS31-008 elongati...	587	2e-164
gi|37519106|gb|AY324744.1|	Stemphylium trifolii EGS12-142 elo...	587	2e-164
gi|37519104|gb|AY324743.1|	Stemphylium sarciniforme EGS38-121...	587	2e-164
gi|37519074|gb|AY324728.1|	Stemphylium sp. EGS49-038 elongati...	581	1e-162
gi|37519038|gb|AY324710.1|	Stemphylium majusculum EGS16-068 e...	581	1e-162
gi|37518986|gb|AY324684.1|	Stemphylium sp. EGS29-062 elongati...	581	1e-162
gi|37518984|gb|AY324683.1|	Stemphylium sp. EGS27-1942 elongat...	581	1e-162
gi|37518982|gb|AY324682.1|	Stemphylium sp. EGS27-1941 elongat...	581	1e-162
gi|37518980|gb|AY324681.1|	Stemphylium sp. EGS08-174 elongati...	581	1e-162
gi|643454|gb|U19723.1|APU19723	Aureobasidium pullulans transl...	579	5e-162
gi|37519100|gb|AY324741.1|	Stemphylium sp. EGS49-054 elongati...	573	3e-160
gi|37519096|gb|AY324739.1|	Stemphylium sp. EGS49-052 elongati...	573	3e-160
gi|37519092|gb|AY324737.1|	Stemphylium sp. EGS49-050 elongati...	573	3e-160
gi|37519086|gb|AY324734.1|	Stemphylium sp. EGS49-046 elongati...	573	3e-160
gi|37519084|gb|AY324733.1|	Stemphylium sp. EGS49-045 elongati...	573	3e-160
gi|37519080|gb|AY324731.1|	Stemphylium sp. EGS49-042 elongati...	573	3e-160
gi|37519078|gb|AY324730.1|	Stemphylium sp. EGS49-041 elongati...	573	3e-160
gi|37519072|gb|AY324727.1|	Stemphylium sp. EGS49-035 elongati...	573	3e-160
gi|37519070|gb|AY324726.1|	Stemphylium sp. EGS49-030 elongati...	573	3e-160
gi|37519064|gb|AY324723.1|	Stemphylium sp. EGS48-175 elongati...	573	3e-160
gi|37519062|gb|AY324722.1|	Stemphylium sp. EGS48-173 elongati...	573	3e-160
gi|37519060|gb|AY324721.1|	Stemphylium sp. EGS48-172 elongati...	573	3e-160
gi|37519058|gb|AY324720.1|	Stemphylium sp. EGS48-171 elongati...	573	3e-160
gi|37519054|gb|AY324718.1|	Stemphylium sp. EGS48-169 elongati...	573	3e-160
gi|37519052|gb|AY324717.1|	Stemphylium sp. EGS48-168 elongati...	573	3e-160
gi|37519050|gb|AY324716.1|	Stemphylium sp. EGS48-167 elongati...	573	3e-160
gi|37519048|gb|AY324715.1|	Stemphylium sp. EGS48-165 elongati...	573	3e-160
gi|37519044|gb|AY324713.1|	Pleospora sedicola EGS48-095 elong...	573	3e-160
gi|37519040|gb|AY324711.1|	Pleospora tomatonis EGS29-089 elon...	573	3e-160
gi|37519036|gb|AY324709.1|	Pleospora gracilariae EGS37-073 el...	573	3e-160
gi|37519034|gb|AY324708.1|	Pleospora sp. EGS37-067 elongation...	573	3e-160
gi|37519032|gb|AY324707.1|	Stemphylium sp. EGS35-187 elongati...	573	3e-160
gi|37519026|gb|AY324704.1|	Stemphylium sp. EGS35-169 elongati...	573	3e-160
gi|37519024|gb|AY324703.1|	Stemphylium sp. EGS35-163 elongati...	573	3e-160
gi|37519022|gb|AY324702.1|	Stemphylium sp. EGS47-135 elongati...	573	3e-160
gi|37519018|gb|AY324700.1|	Stemphylium sp. EGS44-149 elongati...	573	3e-160
gi|37519016|gb|AY324699.1|	Stemphylium sp. EGS42-022 elongati...	573	3e-160
gi|37519012|gb|AY324697.1|	Stemphylium sp. EGS40-038 elongati...	573	3e-160
gi|37519010|gb|AY324696.1|	Stemphylium sp. EGS38-091 elongati...	573	3e-160
gi|37519008|gb|AY324695.1|	Stemphylium sp. EGS38-089 elongati...	573	3e-160
gi|37519006|gb|AY324694.1|	Stemphylium sp. EGS35-190 elongati...	573	3e-160
gi|37519002|gb|AY324692.1|	Stemphylium sp. EGS48-104 elongati...	573	3e-160
gi|37519000|gb|AY324691.1|	Stemphylium sp. EGS48-102 elongati...	573	3e-160
gi|37518996|gb|AY324689.1|	Stemphylium sp. EGS48-099 elongati...	573	3e-160
gi|37518994|gb|AY324688.1|	Stemphylium sp. EGS48-087 elongati...	573	3e-160
gi|37518992|gb|AY324687.1|	Stemphylium sp. EGS48-075 elongati...	573	3e-160
gi|37518988|gb|AY324685.1|	Stemphylium sp. EGS30-181 elongati...	573	3e-160

Appendix B

B.2. Results of the BLAST nucleotide-nucleotide similarity search

3. *D. arenaria* type strains (CBS 181.58) – *rpb2* gene sequence

Sequences producing significant alignments:	Score (Bits)	E Value
gi|6606126|gb|AF107804.1|AF107804 Pleospora herbarum DNA-depe...	1109	0.0
gi|82706470|gb|DQ247794.1| Pleospora herbarum isolate AFTOL-I...	865	0.0
gi|44889501|gb|AY533025.1| Cochliobolus heterostrophus RNA po...	699	0.0
gi|6606124|gb|AF107803.1|AF107803 Curvularia brachyspora DNA-...	644	0.0
gi|82706462|gb|DQ247790.1| Cochliobolus heterostrophus isolat...	568	1e-158
gi|82621734|gb|DQ278491.1| Phaeosphaeria nodorum second large...	259	1e-65
gi|45545338|gb|AY485628.1| Opegrapha varia DNA-dependent RNA ...	224	3e-55
gi|6606128|gb|AF107805.1|AF107805 Sporormiella minima DNA-dep...	220	5e-54
gi|6606120|gb|AF107801.1|AF107801 Mycosphaerella citrullina D...	190	6e-45
gi|6606122|gb|AF107802.1|AF107802 Botryosphaeria rhodina DNA-...	176	1e-40
gi|59894422|gb|AY780193.1| Sinosphaeria bambusicola strain SM...	168	3e-38
gi|46142985|gb|AY533830.1| Gibberella moniliformis RNA polymeras	166	1e-37
gi|52699895|gb|AY641084.1| Thelocarpon laureri DNA-dependent ...	160	8e-36
gi|6606116|gb|AF107799.1|AF107799 Aureobasidium pullulans DNA...	158	3e-35
gi|45545334|gb|AY485626.1| Mycosphaerella punctiformis DNA-de...	145	2e-31
gi|77176467|gb|DQ087242.1| Trichoderma longibrachiatum RNA po...	127	5e-26
gi|77176466|gb|DQ087241.1| Hypocrea jecorina RNA polymerase B...	127	5e-26
gi|45545332|gb|AY485625.1| Melanomma radicans DNA-dependent R...	125	2e-25
gi|44921575|gb|AY481588.1| Hypocrea minutispora RNA polymeras...	123	7e-25
gi|46362130|gb|AY391960.1| Podostroma sp. GJS 95-28 RNA polym...	123	7e-25
gi|46362010|gb|AY391900.1| Hypocrea catoptron strain G.J.S. 0...	123	7e-25
gi|45545336|gb|AY485627.1| Scytalidium dimidiatum DNA-depende...	123	7e-25
gi|55583683|gb|AY786055.1| Flammulina velutipes RNA polymeras...	121	3e-24
gi|67904265|ref|XM_677297.1| Aspergillus nidulans FGSC A4 hyp...	121	3e-24
gi|6606104|gb|AF107793.1|AF107793 Emericella nidulans DNA-dep...	121	3e-24
gi|6606098|gb|AF107790.1|AF107790 Podospora anserina DNA-depe...	121	3e-24
gi|59894408|gb|AY780186.1| Podospora appendiculata strain CBS...	119	1e-23
gi|52699899|gb|AY641086.1| Thelocarpon laureri DNA-dependent ...	117	5e-23
gi|33333659|gb|AF545556.1| Trichoderma strigosum strain DAOM ...	117	5e-23
gi|83415482|gb|DQ302784.1| Mortierella verticillata isolate A...	117	5e-23
gi|33333665|gb|AF545559.1| Hypocrea pulvinata strain GJS 98-1...	115	2e-22
gi|46362120|gb|AY391955.1| Hypocrea tawa strain G.J.S. 02-79 ...	115	2e-22
gi|46362098|gb|AY391944.1| Hypocrea spinulosa strain G.J.S. 9...	115	2e-22
gi|46362060|gb|AY391925.1| Hypocrea lixii strain G.J.S. 90-22...	115	2e-22
gi|14581432|gb|AY015636.1| Hypocrea pallida GJS89-83 RNA poly...	115	2e-22
gi|52699803|gb|AY641037.1| Dibaeis baeomyces DNA-dependent RN...	113	8e-22
gi|45545326|gb|AY485622.1| Dibaeis baeomyces DNA-dependent RN...	113	8e-22
gi|45120563|gb|AY218474.2| Bondarzewia montana isolate AFTOL-...	113	8e-22
gi|6606138|gb|AF107810.1|AF107810 Morchella elata DNA-depende...	113	8e-22
gi|59894424|gb|AY780194.1| Sordaria fimicola RNA polymerase I...	113	8e-22
gi|59894406|gb|AY780185.1| Neurospora pannonica strain TRTC51...	113	8e-22
gi|59894390|gb|AY780177.1| Gelasinospora tetrasperma strain A...	113	8e-22
gi|78100229|gb|DQ234550.1| Colacogloea peniophorae isolate AF...	111	3e-21
gi|33333677|gb|AF545565.1| Trichoderma citrinoviride strain G...	111	3e-21
gi|46111554|ref|XM_382835.1| Gibberella zeae PH-1 chromosome ...	111	3e-21
gi|33333643|gb|AF545548.1| Trichoderma hamatum strain DAOM 16...	109	1e-20
gi|6606108|gb|AF107795.1|AF107795 Trichophyton rubrum DNA-dep...	109	1e-20
gi|33333669|gb|AF545561.1| Hypocrea citrina strain CBS 894.85...	107	5e-20
gi|33333661|gb|AF545557.1| Trichoderma tomentosum strain DAOM...	107	5e-20
gi|33333633|gb|AF545543.1| Hypocrea crassa strain GJS 95-157 ...	107	5e-20
gi|33333631|gb|AF545542.1| Hypocrea crassa strain DAOM 164916...	107	5e-20

Appendix B

B.2. Results of the BLAST nucleotide-nucleotide similarity search

3. *D. arenaria* type strains (CBS 181.58) – *rpb2* gene sequence, cont.

Sequences producing significant alignments:		Score (Bits)	E Value
gi|33333629|gb|AF545541.1|	Trichoderma aggressivum strain CBS...	107	5e-20
gi|33333625|gb|AF545539.1|	Trichoderma stromaticum strain PC ...	107	5e-20
gi|33333585|gb|AF545519.1|	Hypocrea pilulifera strain CBS 814...	107	5e-20
gi|44921577|gb|AY481589.1|	Hypocrea pachybasioides RNA polyme...	107	5e-20
gi|44921573|gb|AY481587.1|	Hypocrea crassa RNA polymerase II ...	107	5e-20
gi|46362122|gb|AY391956.1|	Hypocrea tawa strain G.J.S. 97-174...	107	5e-20
gi|46362100|gb|AY391945.1|	Hypocrea straminea strain G.J.S. 0...	107	5e-20
gi|77176465|gb|DQ087240.1|	Trichoderma rossicum RNA polymeras...	107	5e-20
gi|33333671|gb|AF545562.1|	Hypocrea avellanea strain CTR 77-1...	105	2e-19
gi|23894202|emb|AJ430621.1|TSC430621	Trichophyton schoenleini...	105	2e-19
gi|23894198|emb|AJ430620.1|TME430620	Trichophyton mentagrophy...	105	2e-19
gi|84313733|gb|DQ315052.1|	Porpidia melinodes isolate Pormel2...	103	9e-19
gi|84313689|gb|DQ315030.1|	Porpidia melinodes isolate Pormel1...	103	9e-19
gi|59894394|gb|AY780179.1|	Lasiosphaeria hirsuta strain SMH15...	103	9e-19
gi|77176469|gb|DQ087243.1|	Trichoderma saturnisporum RNA poly...	103	9e-19
gi|33333675|gb|AF545564.1|	Hypocrea pezizoides strain GJS 01-...	99.5	1e-17
gi|33333663|gb|AF545558.1|	Hypocrea virens strain GLi 39 RNA ...	99.5	1e-17
gi|33333657|gb|AF545555.1|	Hypocrea strictipilis strain DAOM ...	99.5	1e-17
gi|33333655|gb|AF545554.1|	Hypocrea strictipilis strain DAOM ...	99.5	1e-17
gi|33333641|gb|AF545547.1|	Gliocladium flavofuscum strain DAO...	99.5	1e-17
gi|33333635|gb|AF545544.1|	Hypocrea strictipilis strain DAOM ...	99.5	1e-17
gi|33333623|gb|AF545538.1|	Hypocrea strictipilis strain GJS 9...	99.5	1e-17
gi|33333621|gb|AF545537.1|	Hypocrea strictipilis strain GJS 9...	99.5	1e-17
gi|33333619|gb|AF545536.1|	Hypocrea strictipilis strain GJS 9...	99.5	1e-17
gi|33333617|gb|AF545535.1|	Hypocrea strictipilis strain GJS 9...	99.5	1e-17
gi|33333613|gb|AF545533.1|	Hypocrea strictipilis strain GJS 9...	99.5	1e-17
gi|33333611|gb|AF545532.1|	Hypocrea strictipilis strain GJS 9...	99.5	1e-17
gi|33333609|gb|AF545531.1|	Hypocrea strictipilis strain GJS 9...	99.5	1e-17
gi|33333607|gb|AF545530.1|	Hypocrea strictipilis strain GJS 9...	99.5	1e-17
gi|33333605|gb|AF545529.1|	Hypocrea strictipilis strain GJS 9...	99.5	1e-17
gi|33333603|gb|AF545528.1|	Hypocrea strictipilis strain GJS 8...	99.5	1e-17
gi|33333601|gb|AF545527.1|	Hypocrea strictipilis strain GJS 8...	99.5	1e-17
gi|33333599|gb|AF545526.1|	Hypocrea strictipilis strain GJS 0...	99.5	1e-17
gi|33333597|gb|AF545525.1|	Hypocrea strictipilis strain GJS 0...	99.5	1e-17
gi|46362118|gb|AY391954.1|	Hypocrea sulawesensis strain G.J.S...	99.5	1e-17
gi|46362116|gb|AY391953.1|	Hypocrea strictipilosa strain G.J....	99.5	1e-17
gi|46362114|gb|AY391952.1|	Hypocrea strictipilosa strain G.J....	99.5	1e-17
gi|46362110|gb|AY391950.1|	Hypocrea strictipilosa strain G.J....	99.5	1e-17
gi|46362108|gb|AY391949.1|	Hypocrea strictipilosa strain G.J....	99.5	1e-17
gi|46362106|gb|AY391948.1|	Hypocrea strictipilosa strain G.J....	99.5	1e-17
gi|46362104|gb|AY391947.1|	Hypocrea strictipilosa strain G.J....	99.5	1e-17
gi|46362102|gb|AY391946.1|	Hypocrea strictipilosa strain G.J....	99.5	1e-17
gi|46362014|gb|AY391902.1|	Hypocrea ceracea strain G.J.S. 89-...	99.5	1e-17
gi|46362012|gb|AY391901.1|	Hypocrea ceracea strain G.J.S. 88-...	99.5	1e-17
gi|45545314|gb|AY485616.1|	Cenococcum geophilum DNA-dependent...	99.5	1e-17
gi|89274909|gb|DQ408126.1|	Gloeocystidiellum porosum RNA poly...	99.5	1e-17
gi|59894396|gb|AY780180.1|	Lasiosphaeria hispida strain SMH33...	99.5	1e-17
gi|59894378|gb|AY780171.1|	Chaetomium microascoides strain F-...	99.5	1e-17
gi|77176463|gb|DQ087239.1|	Trichoderma helicum RNA polymerase...	99.5	1e-17

Appendix B

B.2. Results of the BLAST nucleotide-nucleotide similarity search

3. *D. salina* strain (TUBs 7892) - *rpb2* gene sequence

Sequences producing significant alignments:	Score (Bits)	E Value
gi|6606126|gb|AF107804.1|AF107804 Pleospora herbarum DNA-depe...	961	0.0
gi|82706470|gb|DQ247794.1| Pleospora herbarum isolate AFTOL-I...	747	0.0
gi|44889501|gb|AY533025.1| Cochliobolus heterostrophus RNA po...	700	0.0
gi|6606124|gb|AF107803.1|AF107803 Curvularia brachyspora DNA-...	601	1e-168
gi|82706462|gb|DQ247790.1| Cochliobolus heterostrophus isolat...	581	1e-162
gi|82621734|gb|DQ278491.1| Phaeosphaeria nodorum second large...	250	5e-63
gi|6606128|gb|AF107805.1|AF107805 Sporormiella minima DNA-dep...	210	4e-51
gi|45545338|gb|AY485628.1| Opegrapha varia DNA-dependent RNA ...	194	2e-46
gi|6606120|gb|AF107801.1|AF107801 Mycosphaerella citrullina D...	194	2e-46
gi|6606116|gb|AF107799.1|AF107799 Aureobasidium pullulans DNA...	168	1e-38
gi|6606122|gb|AF107802.1|AF107802 Botryosphaeria rhodina DNA-...	163	9e-37
gi|46142985|gb|AY533830.1| Gibberella moniliformis RNA polymeras	161	3e-36
gi|59894422|gb|AY780193.1| Sinosphaeria bambusicola strain SM...	159	1e-35
gi|82504134|gb|DQ248316.1| Symbiotaphrina kochii voucher CBS ...	137	5e-29
gi|33333659|gb|AF545556.1| Trichoderma strigosum strain DAOM ...	135	2e-28
gi|45545334|gb|AY485626.1| Mycosphaerella punctiformis DNA-de...	133	8e-28
gi|6606138|gb|AF107810.1|AF107810 Morchella elata DNA-depende...	133	8e-28
gi|52699895|gb|AY641084.1| Thelocarpon laureri DNA-dependent ...	131	3e-27
gi|83415482|gb|DQ302784.1| Mortierella verticillata isolate A...	125	2e-25
gi|44921575|gb|AY481588.1| Hypocrea minutispora RNA polymeras...	121	3e-24
gi|46362130|gb|AY391960.1| Podostroma sp. GJS 95-28 RNA polym...	121	3e-24
gi|45545314|gb|AY485616.1| Cenococcum geophilum DNA-dependent...	121	3e-24
gi|33333633|gb|AF545543.1| Hypocrea crassa strain GJS 95-157 ...	119	1e-23
gi|33333631|gb|AF545542.1| Hypocrea crassa strain DAOM 164916...	119	1e-23
gi|33333589|gb|AF545521.1| Hypocrea rufa strain GJS 89-127 RN...	119	1e-23
gi|46362098|gb|AY391944.1| Hypocrea spinulosa strain G.J.S. 9...	119	1e-23
gi|46362010|gb|AY391900.1| Hypocrea catoptron strain G.J.S. 0...	119	1e-23
gi|6606108|gb|AF107795.1|AF107795 Trichophyton rubrum DNA-dep...	119	1e-23
gi|6606098|gb|AF107790.1|AF107790 Podospora anserina DNA-depe...	117	5e-23
gi|45545332|gb|AY485625.1| Melanomma radicans DNA-dependent R...	115	2e-22
gi|83773738|dbj|AP007169.1| Aspergillus oryzae RIB40 genomic DNA	115	2e-22
gi|59894408|gb|AY780186.1| Podospora appendiculata strain CBS...	115	2e-22
gi|77176467|gb|DQ087242.1| Trichoderma longibrachiatum RNA po...	115	2e-22
gi|77176466|gb|DQ087241.1| Hypocrea jecorina RNA polymerase B...	115	2e-22
gi|23894202|emb|AJ430621.1|TSC430621 Trichophyton schoenleini...	115	2e-22
gi|23894198|emb|AJ430620.1|TME430620 Trichophyton mentagrophy...	115	2e-22
gi|33333661|gb|AF545557.1| Trichoderma tomentosum strain DAOM...	113	7e-22
gi|77176462|gb|DQ087238.1| Hypocrea pachybasioides RNA polyme...	113	7e-22
gi|55583683|gb|AY786055.1| Flammulina velutipes RNA polymeras...	111	3e-21
gi|46362120|gb|AY391955.1| Hypocrea tawa strain G.J.S. 02-79 ...	111	3e-21
gi|46362060|gb|AY391925.1| Hypocrea lixii strain G.J.S. 90-22...	111	3e-21
gi|59894430|gb|AY780197.1| Triangularia tanzaniensis strain T...	111	3e-21
gi|52699899|gb|AY641086.1| Thelocarpon laureri DNA-dependent ...	109	1e-20
gi|52699803|gb|AY641037.1| Dibaeis baeomyces DNA-dependent RN...	109	1e-20
gi|67904265|ref|XM_677297.1| Aspergillus nidulans FGSC A4 hyp...	109	1e-20
gi|45545326|gb|AY485622.1| Dibaeis baeomyces DNA-dependent RN...	109	1e-20
gi|6606104|gb|AF107793.1|AF107793 Emericella nidulans DNA-dep...	109	1e-20
gi|59894352|gb|AY780158.1| Catabotrys deciduum strain SMH3436...	109	1e-20
gi|33333643|gb|AF545548.1| Trichoderma hamatum strain DAOM 16...	105	2e-19
gi|46362044|gb|AY391917.1| Hypocrea cinnamomea strain G.J.S. ...	105	2e-19

Appendix B

B.2. Results of the BLAST nucleotide-nucleotide similarity search

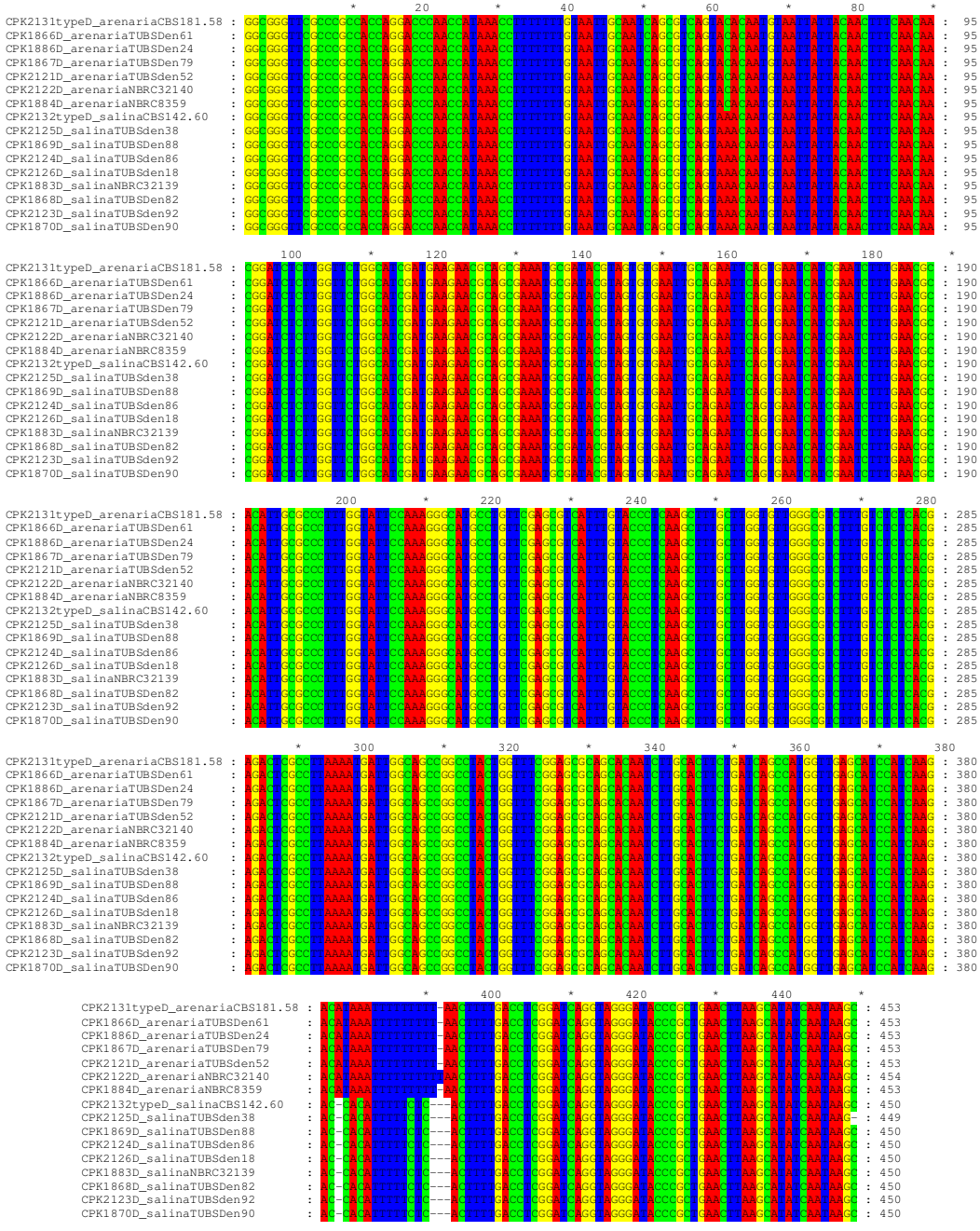
3. *D. salina* strain (TUBs 7892) - *rpb*2 gene sequence, cont.

Sequences producing significant alignments:		Score (Bits)	E Value
gi|46362042|gb|AY391916.1|	Hypocrea cinnamomea strain G.J.S. ...	105	2e-19
gi|33333675|gb|AF545564.1|	Hypocrea pezizoides strain GJS 01-...	103	7e-19
gi|33333665|gb|AF545559.1|	Hypocrea pulvinata strain GJS 98-1...	103	7e-19
gi|33333657|gb|AF545555.1|	Hypocrea strictipilis strain DAOM ...	103	7e-19
gi|33333655|gb|AF545554.1|	Hypocrea strictipilis strain DAOM ...	103	7e-19
gi|33333653|gb|AF545553.1|	Trichoderma spirale strain DAOM 18...	103	7e-19
gi|33333635|gb|AF545544.1|	Hypocrea strictipilis strain DAOM ...	103	7e-19
gi|33333629|gb|AF545541.1|	Trichoderma aggressivum strain CBS...	103	7e-19
gi|33333625|gb|AF545539.1|	Trichoderma stromaticum strain PC ...	103	7e-19
gi|33333623|gb|AF545538.1|	Hypocrea strictipilis strain GJS 9...	103	7e-19
gi|33333621|gb|AF545537.1|	Hypocrea strictipilis strain GJS 9...	103	7e-19
gi|33333619|gb|AF545536.1|	Hypocrea strictipilis strain GJS 9...	103	7e-19
gi|33333617|gb|AF545535.1|	Hypocrea strictipilis strain GJS 9...	103	7e-19
gi|33333613|gb|AF545533.1|	Hypocrea strictipilis strain GJS 9...	103	7e-19
gi|33333611|gb|AF545532.1|	Hypocrea strictipilis strain GJS 9...	103	7e-19
gi|33333609|gb|AF545531.1|	Hypocrea strictipilis strain GJS 9...	103	7e-19
gi|33333607|gb|AF545530.1|	Hypocrea strictipilis strain GJS 9...	103	7e-19
gi|33333605|gb|AF545529.1|	Hypocrea strictipilis strain GJS 9...	103	7e-19
gi|33333603|gb|AF545528.1|	Hypocrea strictipilis strain GJS 8...	103	7e-19
gi|33333601|gb|AF545527.1|	Hypocrea strictipilis strain GJS 8...	103	7e-19
gi|33333599|gb|AF545526.1|	Hypocrea strictipilis strain GJS 0...	103	7e-19
gi|33333597|gb|AF545525.1|	Hypocrea strictipilis strain GJS 0...	103	7e-19
gi|33333595|gb|AF545524.1|	Hypocrea strictipilis strain CTR 7...	103	7e-19
gi|33333593|gb|AF545523.1|	Hypocrea strictipilis strain CTR 7...	103	7e-19
gi|33333585|gb|AF545519.1|	Hypocrea pilulifera strain CBS 814...	103	7e-19
gi|44921577|gb|AY481589.1|	Hypocrea pachybasioides RNA polyme...	103	7e-19
gi|44921573|gb|AY481587.1|	Hypocrea crassa RNA polymerase II ...	103	7e-19
gi|46362122|gb|AY391956.1|	Hypocrea tawa strain G.J.S. 97-174...	103	7e-19
gi|46362118|gb|AY391954.1|	Hypocrea sulawesensis strain G.J.S...	103	7e-19
gi|46362116|gb|AY391953.1|	Hypocrea strictipilosa strain G.J....	103	7e-19
gi|46362114|gb|AY391952.1|	Hypocrea strictipilosa strain G.J....	103	7e-19
gi|46362110|gb|AY391950.1|	Hypocrea strictipilosa strain G.J....	103	7e-19
gi|46362108|gb|AY391949.1|	Hypocrea strictipilosa strain G.J....	103	7e-19
gi|46362106|gb|AY391948.1|	Hypocrea strictipilosa strain G.J....	103	7e-19
gi|46362104|gb|AY391947.1|	Hypocrea strictipilosa strain G.J....	103	7e-19
gi|46362102|gb|AY391946.1|	Hypocrea strictipilosa strain G.J....	103	7e-19
gi|46362100|gb|AY391945.1|	Hypocrea straminea strain G.J.S. 0...	103	7e-19
gi|46362050|gb|AY391920.1|	Hypocrea cinnamomea strain G.J.S. ...	103	7e-19
gi|46362048|gb|AY391919.1|	Hypocrea cinnamomea strain G.J.S. ...	103	7e-19
gi|46362046|gb|AY391918.1|	Hypocrea cinnamomea strain G.J.S. ...	103	7e-19
gi|59894396|gb|AY780180.1|	Lasiosphaeria hispida strain SMH33...	103	7e-19
gi|14581432|gb|AY015636.1|	Hypocrea pallida GJS89-83 RNA poly...	103	7e-19
gi|77176465|gb|DQ087240.1|	Trichoderma rossicum RNA polymeras...	103	7e-19
gi|52699819|gb|AY641045.1|	Gyromitra esculenta DNA-dependent ...	101	3e-18
gi|46111554|ref|XM_382835.1|	Gibberella zeae PH-1 chromosome ...	101	3e-18
gi|6606110|gb|AF107796.1|AF107796	Exophiala jeanselmei DNA-de...	101	3e-18
gi|59894424|gb|AY780194.1|	Sordaria fimicola RNA polymerase I...	101	3e-18
gi|59894406|gb|AY780185.1|	Neurospora pannonica strain TRTC51...	101	3e-18
gi|59894364|gb|AY780164.1|	Cercophora macrocarpa strain SMH20...	101	3e-18
gi|84313733|gb|DQ315052.1|	Porpidia melinodes isolate Pormel2...	99.6	1e-17

Appendix B

B.3. Sequence alignments of *Dendryphiella* and *Scolecobasidium* species

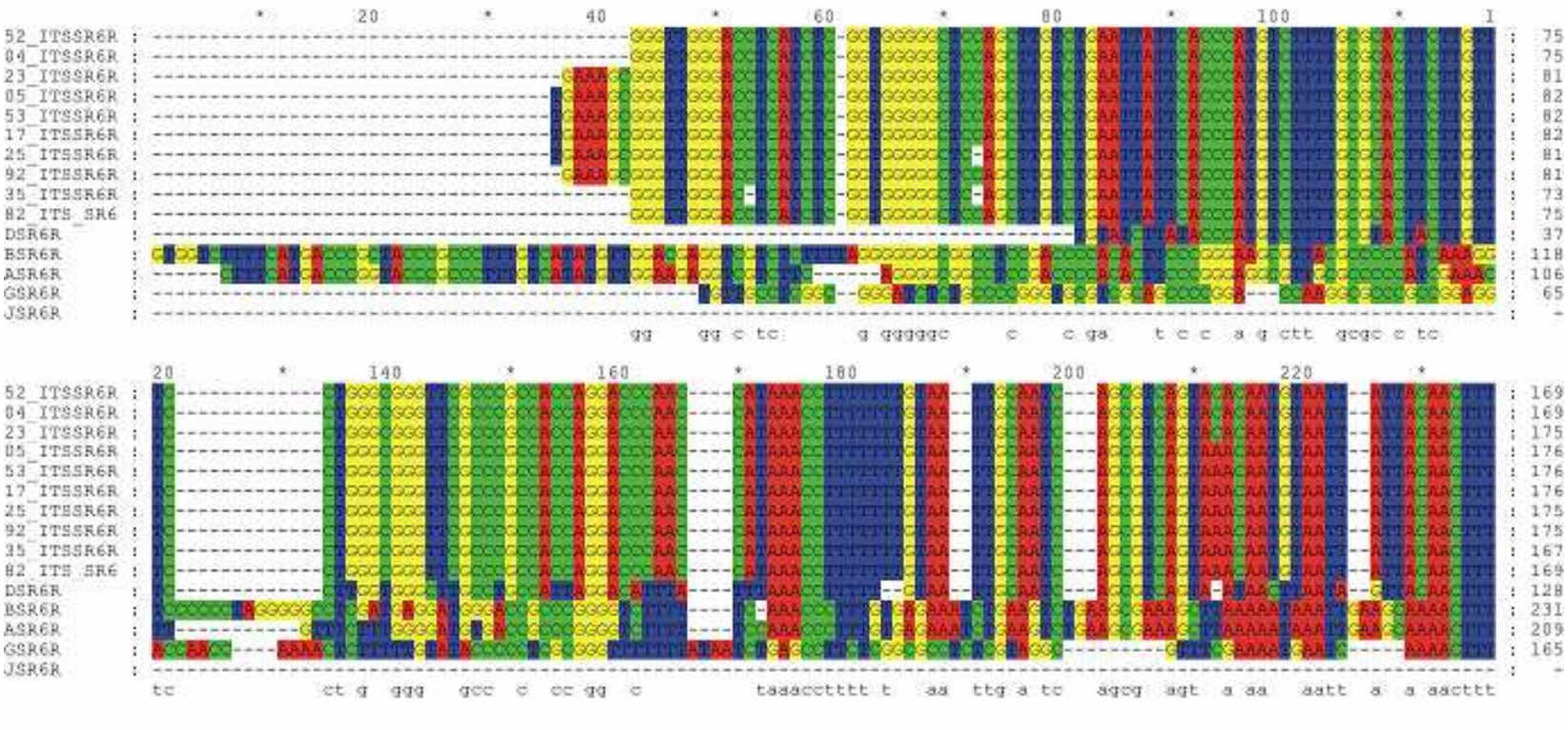
1. ITS sequence alignment of isolated *Dendryphiella* strains with *D. arenaria* type strains (CBS 181.58) and *D. salina* reference strain (CBS 142.50)



Appendix B

B.3. Sequence alignments of *Dendryphiella* and *Scolecobasidium* species

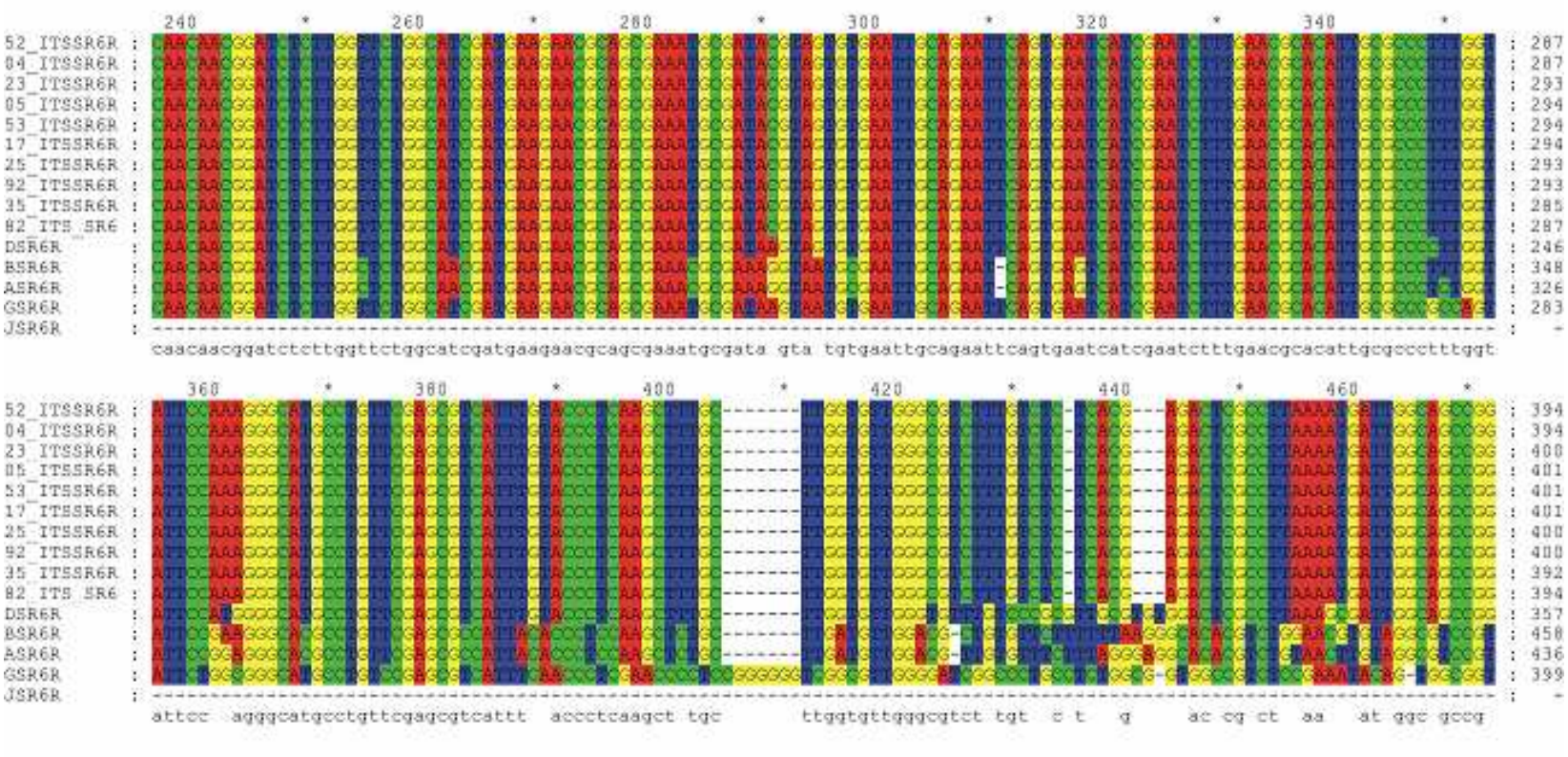
2. ITS sequence alignment of *Dendryphiella* (as numbers) and *Scolecobasidium* (as letters) species



Appendix B

B.3. Sequence alignments of *Dendryphiella* and *Scolecobasidium* species

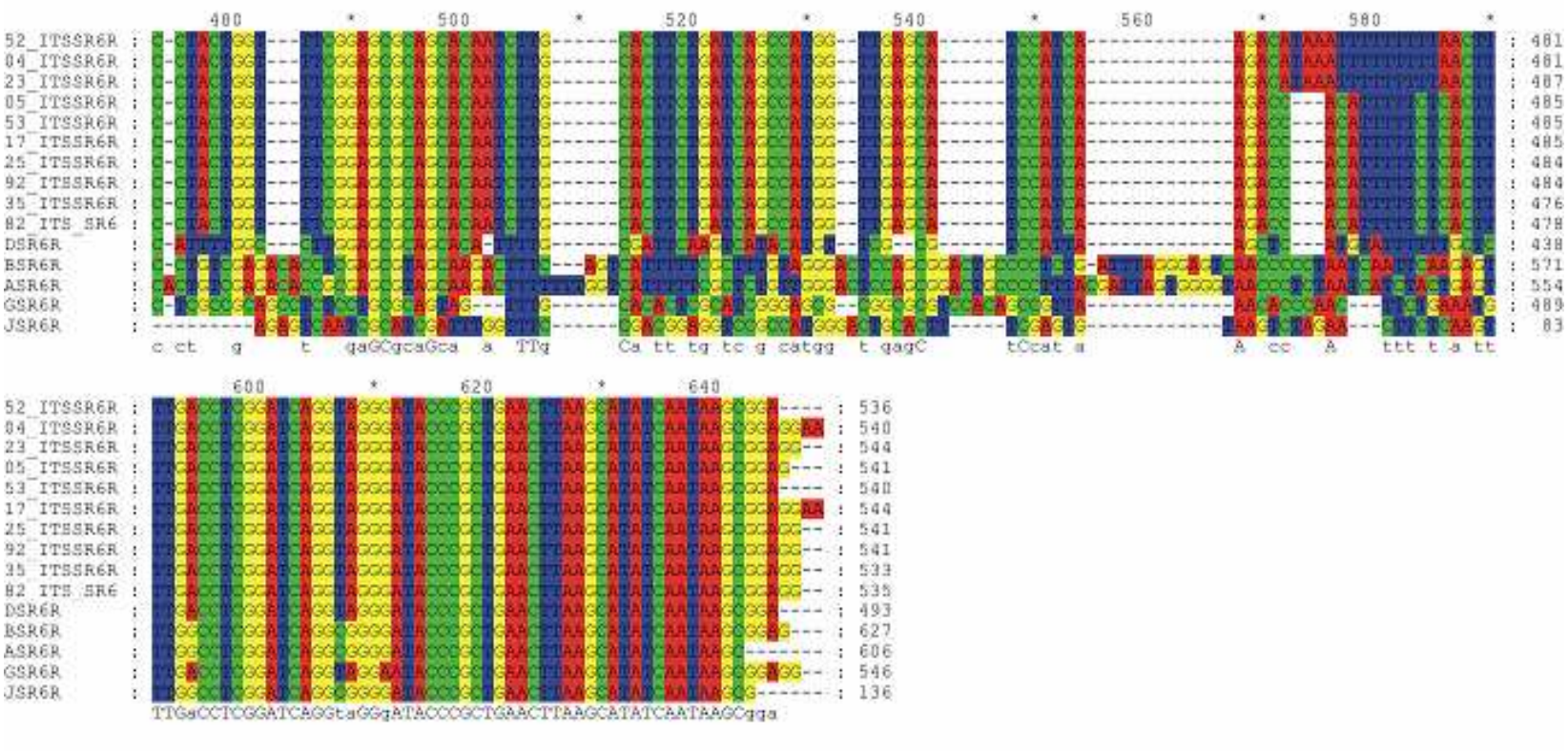
2. ITS sequence alignment of *Dendryphiella* (as numbers) and *Scolecobasidium* (as letters) species



Appendix B

B.3. Sequence alignments of *Dendryphiella* and *Scolecobasidium* species

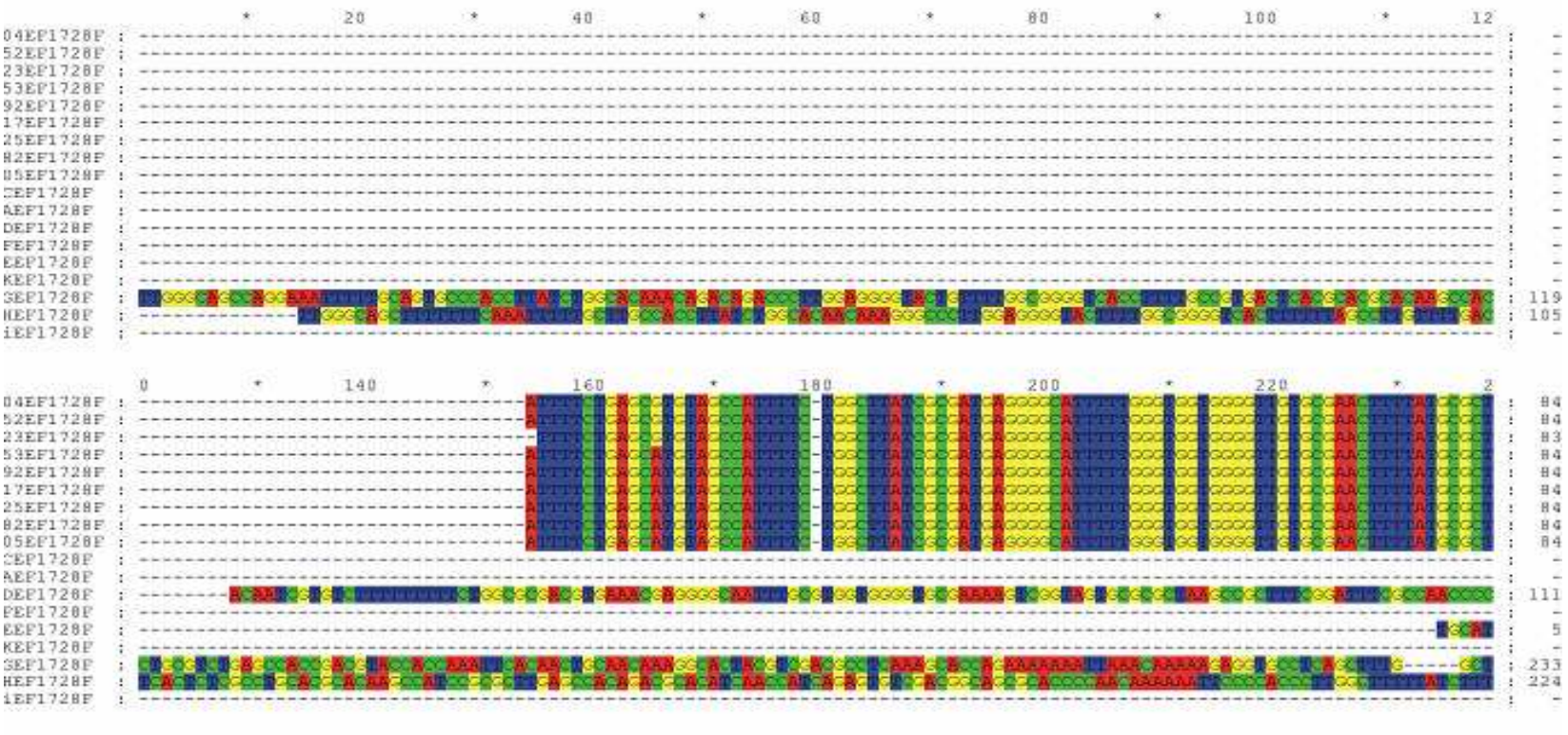
2. ITS sequence alignment of *Dendryphiella* (as numbers) and *Scolecobasidium* (as letters) species



Appendix B

B.3. Sequence alignments of *Dendryphiella* and *Scolecobasidium* species

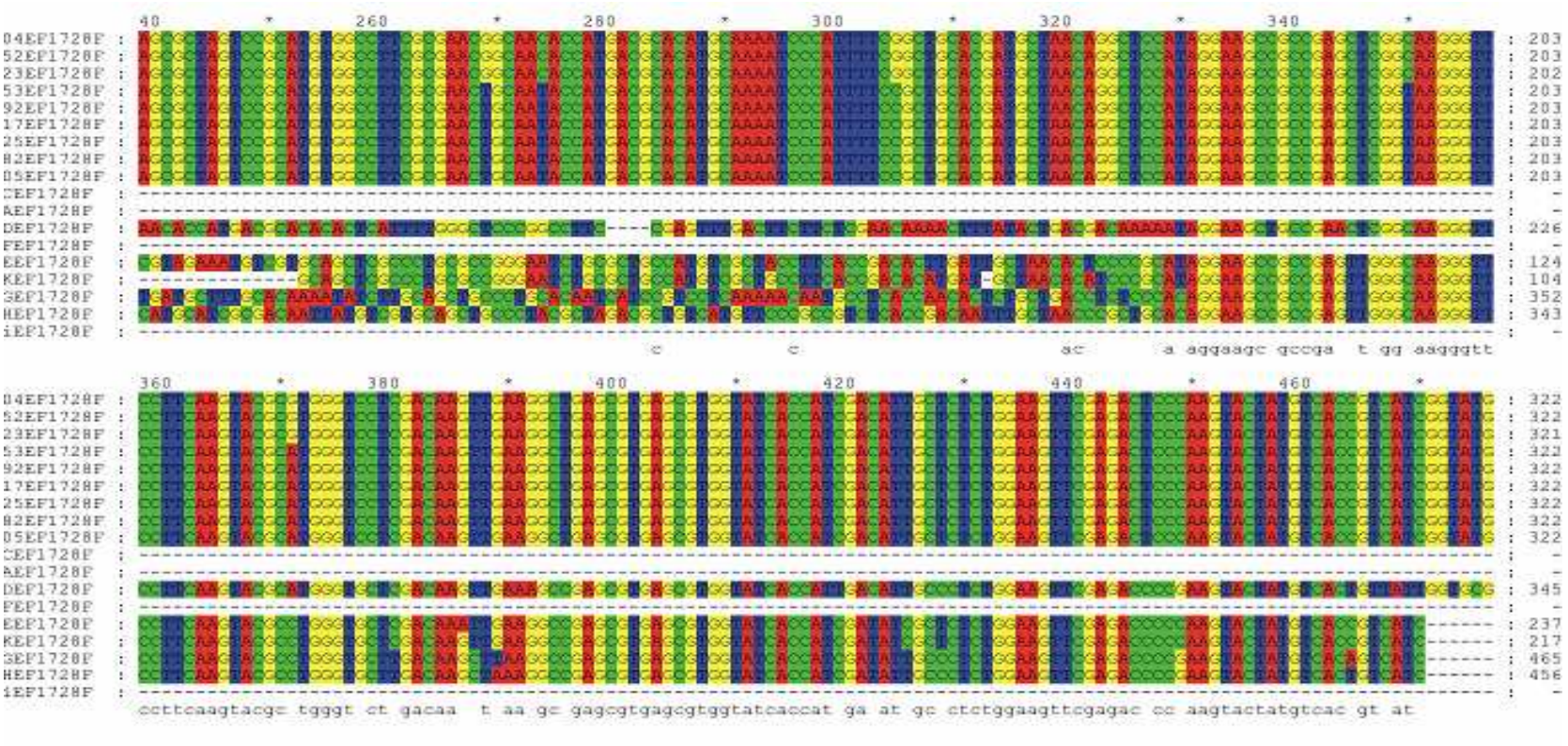
3. *tef*1 sequence alignment of *Dendryphiella* (as numbers) and *Scolecobasidium* (as letters) species



Appendix B

B.3. Sequence alignments of *Dendryphiella* and *Scolecobasidium* species

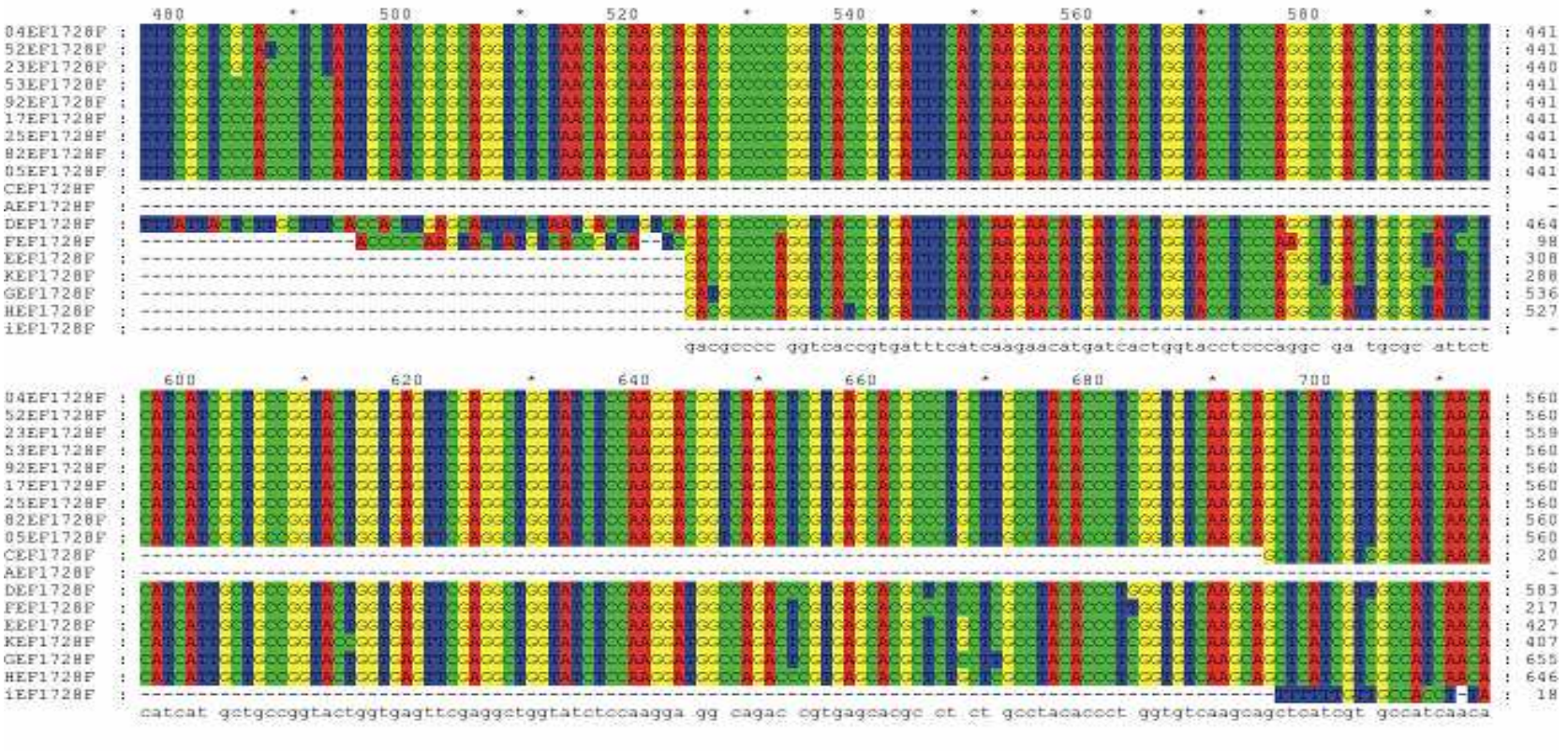
3. *tef*1 sequence alignment of *Dendryphiella* (as numbers) and *Scolecobasidium* (as letters) species



Appendix B

B.3. Sequence alignments of *Dendryphiella* and *Scolecobasidium* species

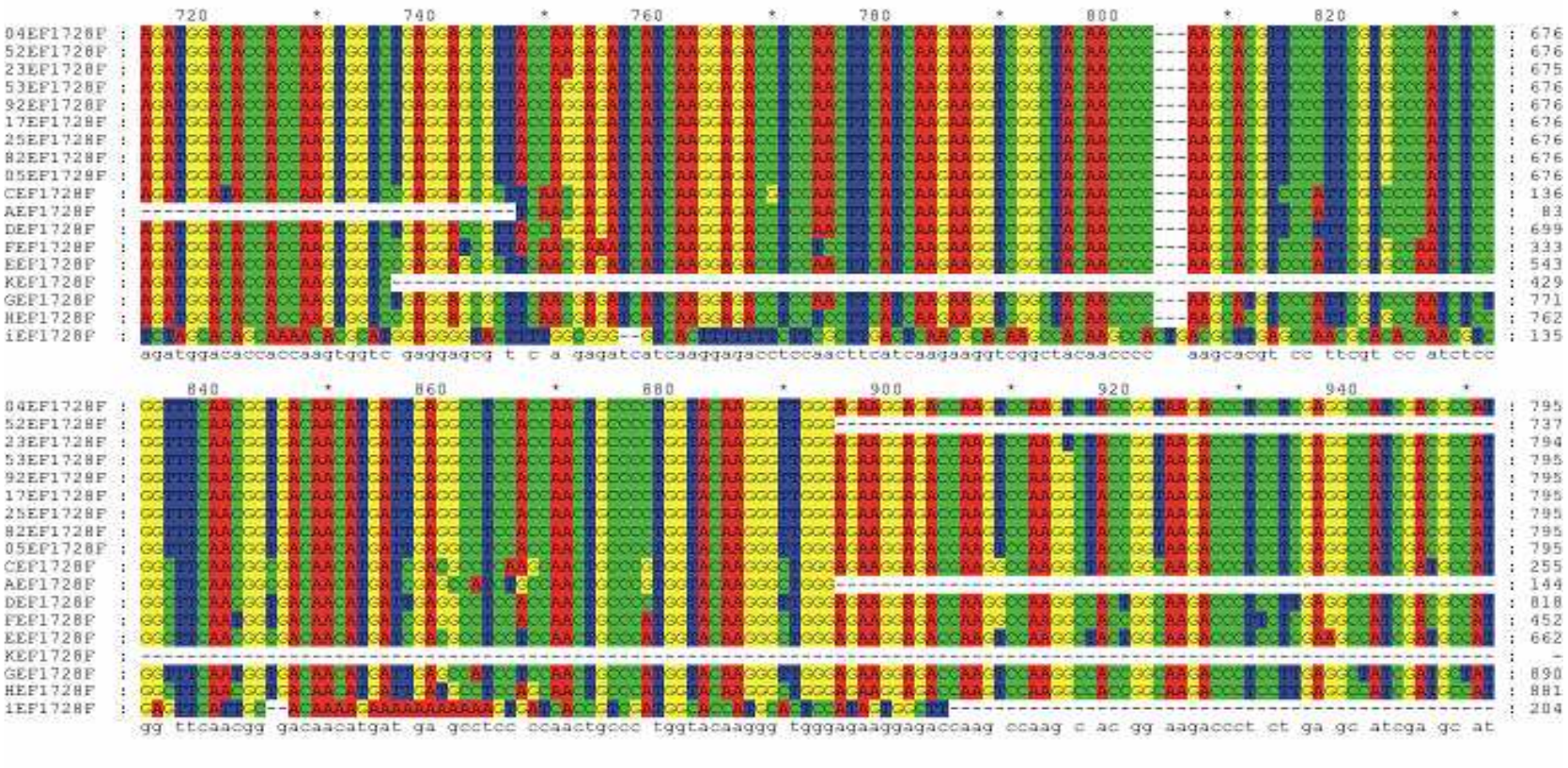
3. *tef*1 sequence alignment of *Dendryphiella* (as numbers) and *Scolecobasidium* (as letters) species



Appendix B

B.3. Sequence alignments of *Dendryphiella* and *Scolecobasidium* species

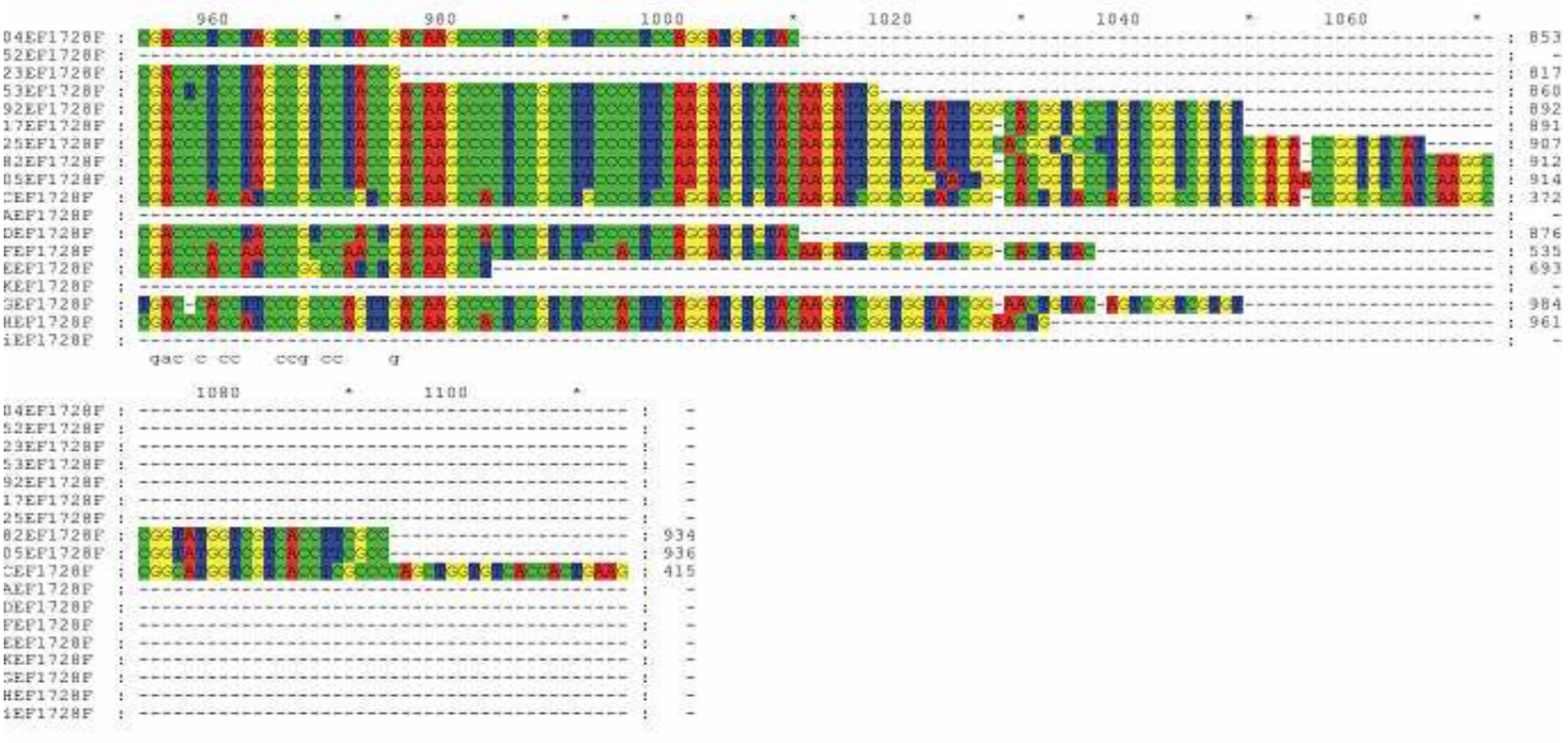
3. *tef*1 sequence alignment of *Dendryphiella* (as numbers) and *Scolecobasidium* (as letters) species



Appendix B

B.3. Sequence alignments of *Dendryphiella* and *Scolecobasidium* species

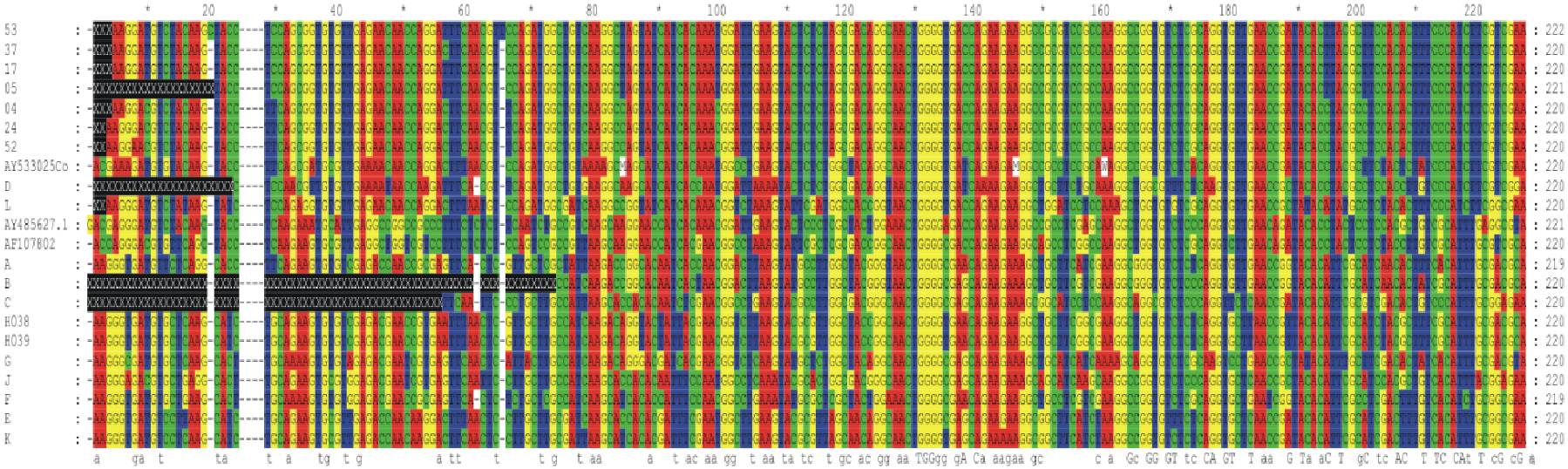
3. *tef*1 sequence alignment of *Dendryphiella* (as numbers) and *Scolecobasidium* (as letters) species



Appendix B

B.3. Sequence alignments of *Dendryphiella* and *Scolecobasidium* species

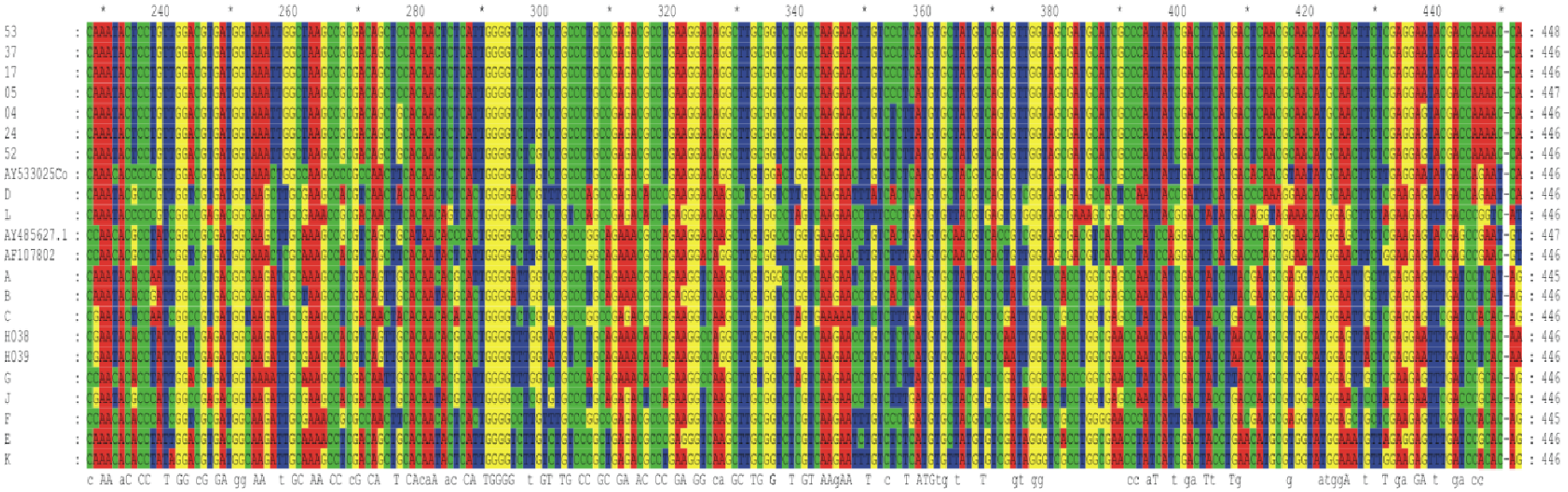
4. *rpb2* sequence alignment of *Dendryphiella* (as numbers) and *Scolecobasidium* (as letters) species



Appendix B

B.3. Sequence alignments of *Dendryphiella* and *Scolecobasidium* species

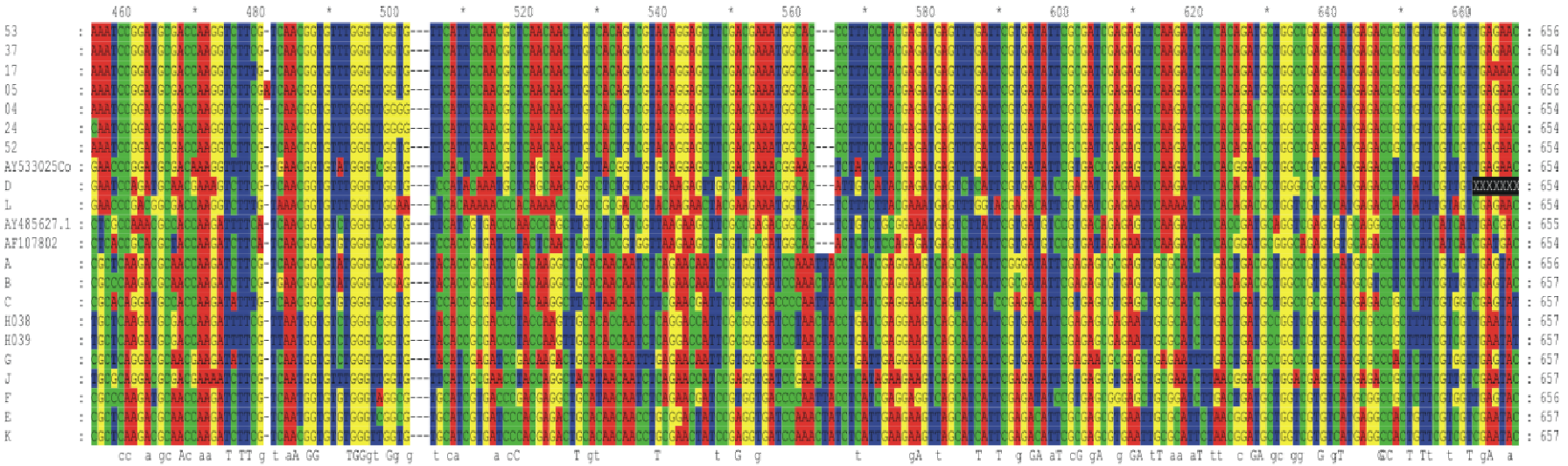
4. *rpb2* sequence alignment of *Dendryphiella* (as numbers) and *Scolecobasidium* (as letters) species



Appendix B

B.3. Sequence alignments of *Dendryphiella* and *Scolecobasidium* species

4. *rpb2* sequence alignment of *Dendryphiella* (as numbers) and *Scolecobasidium* (as letters) species



Appendix C

Morphocultural characters of the individual strains of *D. arenaria* and *D. salina*

C. 1. Conidial morphology and colony extension rates

strain nr. [a]	spore dimensions (in µm) [b]			colony extension rates (in cm day^{-1}) [c]	
	length	width	no. of septa	MEAS	PDAS
D. arenaria					
Bs 01 (TUBS 7888)	13 - 32 (20.7 ± 0.4)	5 - 10 (7.1 ± 0.1)	1 - 4	1.46 ± 0.01	**1.59 ± 0.01**
Bs 02 (TUBS 7889)	10 - 29 (18.9 ± 0.3)	5 - 8 (6.6 ± 0.07)	1 - 3	1.74 ± 0.01	**1.81 ± 0.01**
Bs 03 (TUBS 7890)	12 - 33 (20.5 ± 0.4)	5 - 12 (7.2 ± 0.1)	1 - 4	**1.75 ± 0.01**	1.72 ± 0.01
Bs 04 (TUBS 7891)	10 - 33 (18.1 ± 0.4)	5 - 10 (6.8 ± 0.1)	1 - 5	1.80 ± 0.01	**1.85 ± 0.01**
Bs 94 (TUBS 8520)	10 - 32 (17.4 ± 0.4)	5 - 11 (6.8 ± 0.1)	1 - 5	**1.44 ± 0.01**	1.35 ± 0.03
Ms 31 (TUBS 7911)	9 - 26 (14.1 ± 0.3)	4 - 9 (6.1 ± 0.09)	1 - 5	1.55 ± 0.03	1.60 ± 0.03
Ms 36 (TUBS 7916)	10 - 25 (14.4 ± 0.3)	5 - 9 (5.8 ± 0.06)	1 - 3	1.59 ± 0.01	**1.70 ± 0.01**
Jp 50 (NBRC 32140)	11 - 47 (24.7 ± 0.8)	5 - 14 (8.1 ± 0.2)	1 - 6	1.29 ± 0.01	1.33 ± 0.02
Fr 52 (UAMH 1357)	11 - 29 (18.6 ± 0.3)	4 - 7 (5.3 ± 0.08)	1 - 3	1.35 ± 0.01	**1.49 ± 0.02**
Fr 54 (NBRC 8359)	10 - 36 (17.7 ± 0.5)	5 - 9 (6.1 ± 0.1)	1 - 4	1.64 ± 0.02	1.63 ± 0.01
Gm 22 (TUBS 7538)	11 - 31 (18.1 ± 0.4)	5 - 10 (7.0 ± 0.1)	1 - 4	1.39 ± 0.02	1.34 ± 0.08
Gm 23 (TUBS 7527)	10 - 26 (15.3 ± 0.3)	5 - 7 (5.8 ± 0.06)	1 - 3	1.43 ± 0.03	1.49 ± 0.02
Gm 27 (TUBS 7541)	9 - 27 (15.1 ± 0.3)	5 - 9 (6.7 ± 0.08)	1 - 4	**1.30 ± 0.02**	1.03 ± 0.05
Gm 56 (TUBS 8195)	11 - 32 (16.9 ± 0.4)	5 - 10 (6.6 ± 0.1)	1 - 4	1.43 ± 0.02	1.39 ± 0.02
Gm 58 (TUBS 8197)	10 - 35 (16.0 ± 0.4)	5 - 9 (6.8 ± 0.1)	1 - 3	1.49 ± 0.02	1.50 ± 0.03
Gm 61 (TUBS 8200)	10 - 26 (15.6 ± 0.4)	5 - 8 (6.3 ± 0.08)	1 - 3	1.59 ± 0.05	1.59 ± 0.03
Gm 77 (TUBS 8216)	9 - 24 (14.7 ± 0.3)	5 - 8 (6.2 ± 0.08)	1 - 4	1.51 ± 0.01	**1.59 ± 0.03**
Gm 79 (TUBS 8218)	10 - 36 (17.7 ± 0.5)	5 - 9 (6.8 ± 0.1)	1 - 4	1.68 ± 0.01	1.65 ± 0.03
Gm 24 (TUBS 7479)	10 - 27 (15.6 ± 0.3)	5 - 9 (6.4 ± 0.09)	1 - 4	1.39 ± 0.01	**1.43 ± 0.01**
Gm 26 (TUBS 7515)	8 - 26 (14.9 ± 0.3)	5 - 8 (6.0 ± 0.08)	1 - 4	1.17 ± 0.03	1.11 ± 0.02
Gm 28 (TUBS 6551)	12 - 28 (17.5 ± 0.3)	5 - 7 (5.5 ± 0.06)	1 - 3	1.49 ± 0.01	**1.55 ± 0.01**

Appendix C

C. 1. Conidial morphology and colony extension rates

strain nr. [a]	spore dimensions (in µm) [b]			colony extension rates (in cm day^{-1}) [c]	
	length	width	no. of septa	MEAS	PDAS
D. salina					
Bs 05 (TUBS 7892)	12 - 45 (23.9 ± 0.7)	5 - 10 (7.2 ± 0.1)	1 - 7	0.77 ± 0.01	0.79 ± 0.01
Ns 06 (TUBS 7893)	11 - 44 (22.5 ± 0.6)	5 - 10 (7.5 ± 0.1)	1 - 5	1.02 ± 0.01	1.02 ± 0.01
Ns 08 (TUBS 7895)	15 - 67 (27.0 ± 0.9)	5 - 10 (7.1 ± 0.09)	1 - 8	0.89 ± 0.01	**0.93 ± 0.01**
Ns 10 (TUBS 7897)	13 - 72 (26.8 ± 0.9)	5 - 13 (8.1 ± 0.1)	1 - 9	**0.95 ± 0.20**	0.38 ± 0.01
Ns 16 (TUBS 7903)	11 - 37 (20.0 ± 0.6)	5 - 10 (7.1 ± 0.1)	1 - 5	**0.46 ± 0.01**	0.41 ± 0.01
Ns 20 (TUBS 7907)	14 - 44 (23.0 ± 0.6)	5 - 10 (7.0 ± 0.1)	1 - 6	1.13 ± 0.01	**1.16 ± 0.01**
Ns 11 (TUBS 7898)	12 - 40 (23.0 ± 0.6)	6 - 11 (7.1 ± 0.1)	1 - 4	**1.11 ± 0.01**	1.06 ± 0.01
Ns 13 (TUBS 7900)	11 - 51 (24.3 ± 0.9)	5 - 9 (7.0 ± 0.1)	1 - 5	**0.68 ± 0.01**	0.50 ± 0.02
Ns 21 (TUBS 7908)	12 - 53 (27.0 ± 1.0)	6 - 13 (7.8 ± 0.1)	1 - 6	**0.61 ± 0.01**	0.51 ± 0.01
Ns 17 (TUBS 7904)	11 - 43 (23.0 ± 0.8)	5 - 10 (7.2 ± 0.1)	1 - 6	**1.07 ± 0.01**	0.55 ± 0.02
Ns 18 (TUBS 7905)	12 - 42 (23.3 ± 0.8)	5 - 10 (7.3 ± 0.1)	1 - 6	1.09 ± 0.01	1.08 ± 0.03
Ns 19 (TUBS 7906)	12 - 49 (21.4 ± 0.8)	5 - 11 (7.2 ± 0.1)	1 - 5	**0.59 ± 0.01**	0.51 ± 0.01
Ns 45 (TUBS 7925)	11 - 43 (21.7 ± 0.7)	5 - 10 (7.2 ± 0.1)	1 - 6	0.90 ± 0.06	0.76 ± 0.04
Ns 29 (TUBS 7909)	11 - 58 (23.3 ± 0.8)	5 - 12 (7.2 ± 0.1)	1 - 7	**0.73 ± 0.04**	0.57 ± 0.05
Ns 30 (TUBS 7910)	11 - 55 (23.5 ± 0.8)	5 - 11 (7.4 ± 0.1)	1 – 6	**0.84 ± 0.04**	0.55 ± 0.01
Ms 32 (TUBS 7912)	8 - 27 (13.7 ± 0.4)	5 - 9 (6.4 ± 0.09)	1 - 4	0.65 ± 0.01	0.64 ± 0.04
Ms 33 (TUBS 7913)	8 - 28 (15.4 ± 0.5)	5 - 8 (6.3 ± 0.08)	1 - 5	**0.77 ± 0.02**	0.68 ± 0.01
Ms 34 (TUBS 7914)	8 - 33 (14.6 ± 0.5)	5 - 9 (6.4 ± 0.1)	1 - 5	**1.19 ± 0.01**	1.13 ± 0.00
Ms 35 (TUBS 7915)	9 - 34 (16.9 ± 0.6)	5 - 9 (6.9 ± 0.09)	1 – 5	1.07 ± 0.01	**1.14 ± 0.01**
Ms 37 (TUBS 7917)	9 - 29 (15.3 ± 0.4)	5 - 8 (6.3 ± 0.08)	1 - 4	0.90 ± 0.02	**1.00 ± 0.04**
Ms 38 (TUBS 7918)	8 - 25 (13.0 ± 0.4)	4 - 8 (6.1 ± 0.09)	1 - 4	1.20 ± 0.00	1.19 ± 0.01
Jp 51 (NBRC 32139)	13 - 37 (20.5 ± 0.5)	5 - 10 (6.7 ± 0.1)	1 - 4	0.90 ± 0.01	0.90 ± 0.01

Appendix C

C. 1. Conidial morphology and colony extension rates

strain nr. [a]	spore dimensions (in µm) [b]			colony extension rates (in cm day^{-1}) [c]	
	length	width	no. of septa	MEAS	PDAS
D. salina					
Fr 53 (TUBS 8147)	13 - 54 (23.1 ± 0.9)	5 - 12 (7.4 ± 0.1)	1 - 6	**0.69 ± 0.02**	0.49 ± 0.02
Uk 80 (TUBS 8219)	12 - 45 (24.1 ± 0.7)	5 - 10 (7.2 ± 0.1)	1 - 5	0.99 ± 0.01	**1.04 ± 0.00**
Uk 81 (TUBS 8220)	9 - 53 (21.9 ± 0.8)	5 - 10 (7.3 ± 0.1)	1 - 7	**0.82 ± 0.02**	0.71 ± 0.03
Uk 82 (TUBS 8221)	12 - 60 (20.9 ± 0.8)	5 - 10 (7.5 ± 0.1)	1 - 7	0.47 ± 0.01	0.42 ± 0.04
Uk 83 (TUBS 8222)	10 - 49 (24.0 ± 0.8)	5 - 12 (7.1 ± 0.1)	1 - 7	1.09 ± 0.01	**1.15 ± 0.01**
Uk 84 (TUBS8223)	11 - 50 (22.8 ± 0.9)	5 - 12 (7.5 ± 0.1)	1 - 7	**0.41 ± 0.01**	0.28 ± 0.01
Uk 85 (TUBS 8224)	12 - 49 (25.7 ± 0.8)	5 - 12 (7.7 ± 0.1)	1 - 7	1.13 ± 0.01	**1.20 ± 0.01**
Uk 86 (TUBS 8225)	15 - 52 (25.7 ± 0.8)	5 - 11 (7.4 ± 0.1)	1 - 6	**1.06 ± 0.00**	0.98 ± 0.03
Uk 88 (TUBS 8227)	12 - 48 (22.0 ± 0.9)	5 - 10 (7.1 ± 0.1)	1 - 6	**0.84 ± 0.01**	0.79 ± 0.01
Uk 90 (TUBS 8229)	12 - 55 (22.7 ± 0.8)	5 - 9 (7.0 ± 0.1)	1 - 6	**0.66 ± 0.04**	0.53 ± 0.02
Uk 92 (TUBS 8231)	16 - 41 (25.3 ± 0.6)	5 - 10 (7.2 ± 0.1)	1 - 6	1.07 ± 0.01	1.08 ± 0.01
Gm 25 (TUBS 7508)	15 - 45 (23.0 ± 0.6)	5 - 13 (7.2 ± 0.2)	2 - 6	**1.02 ± 0.01**	1.00 ± 0.01

[a] origin of strains

Baltic Sea (Bs), France (Fr), Gulf of Mexico (Gm), Japan (Jp)

Mediterranean Sea (Ms), North Sea (Ns), United Kingdon (Uk)

[b] number in parenthesis indicates the mean spore length and width ± S. E. M., n = 100.

[c] mean colony extension rates ± S. E. M., n = 9. Values in bold were statistically siginificant.

Appendix C

C. 2. Summary of Statistical Analysis (one-way ANOVA)

	DF	SS	MS	F	P	statistically significant difference ?
mean spore length, n = 100						
between *Dendryphiella* strains	54	87584.96	1621.94	40.55	< 0.001	yes
within *D. arenaria* strains	20	13032.56	651.63	38.50	< 0.001	yes
within *D. salina* strains	33	46756.22	1416.86	26.12	< 0.001	yes
mean spore width, n = 100						
between *Dendryphiella* strains	54	2000.08	37.04	30.06	< 0.001	yes
within *D. arenaria* strains	20	831.03	41.55	43.83	< 0.001	yes
within *D. salina* strains	33	618.31	18.74	13.31	< 0.001	yes
mean colony extension rate, n = 9						
MEAS						
between *Dendryphiella* strains	54	64.77	1.20	135.48	< 0.001	yes
within *D. arenaria* strains	20	4.82	0.24	74.21	< 0.001	yes
within *D. salina* strains	33	14.54	0.44	35.79	< 0.001	yes
PDAS						
between *Dendryphiella* strains	54	91.04	1.69	333.71	< 0.001	yes
within *D. arenaria* strains	20	7.54	0.38	53.41	< 0.001	yes
within *D. salina* strains	33	23.94	0.72	190.27	< 0.001	yes

The differences in the mean values among the strains are greater than would be expected by chance; there is a statistically significant difference ($p < 0.001$).

DF = degrees of freedom, SS = Sum of Squares, MS = Mean Squares

Appendix C

C. 2. Summary of Statistical Analysis (one-way ANOVA)

strain	mean colony extension rate				DF	SS	MS	F	P	
	MEAS	SEM	PDAS	SEM						
Bs 01	1.46	0.01	1.59	0.01	1	0.079	0.0790	67.3080	< 0.001	yes
Bs 02	1.74	0.01	1.81	0.01	1	0.022	0.0215	14.1900	0.002	yes
Bs 03	1.75	0.01	1.72	0.01	1	0.006	0.0055	8.5580	0.010	yes
Bs 04	1.80	0.01	1.85	0.01	1	0.009	0.0095	20.6810	< 0.001	yes
Bs 05	0.77	0.01	0.79	0.01	1	0.002	0.0018	1.7220	0.208	no
Bs 94	1.44	0.01	1.35	0.03	1	0.036	0.0364	7.0610	0.017	yes
Fr 52	1.35	0.01	1.49	0.02	1	0.085	0.0851	36.1460	< 0.001	yes
Fr 53	0.69	0.02	0.49	0.02	1	0.182	0.1820	50.3880	< 0.001	yes
Fr 54	1.64	0.02	1.63	0.01	1	0.001	0.0011	0.4720	0.502	no
Gm 22	1.39	0.02	1.34	0.08	1	0.008	0.0085	0.2860	0.600	no
Gm 23	1.43	0.03	1.49	0.02	1	0.019	0.0185	3.2070	0.092	no
Gm 24	1.39	0.01	1.43	0.01	1	0.011	0.0105	12.3190	0.003	yes
Gm 25	1.02	0.01	1.00	0.01	1	0.003	0.0032	7.0980	0.017	yes
Gm 26	1.17	0.03	1.11	0.02	1	0.017	0.0167	4.0470	0.061	no
Gm 27	1.30	0.02	1.03	0.05	1	0.336	0.3360	25.2150	< 0.001	yes
Gm 28	1.49	0.01	1.55	0.01	1	0.017	0.0171	28.6000	< 0.001	yes
Gm 56	1.43	0.02	1.39	0.02	1	0.006	0.0055	0.0000	0.998	no
Gm 58	1.49	0.02	1.50	0.03	1	0.000	0.0005	0.0702	0.792	no
Gm 61	1.59	0.05	1.59	0.03	1	0.000	0.0000	0.0000	1.000	no
Gm 77	1.51	0.01	1.59	0.03	1	0.027	0.0265	7.5280	0.014	yes
Gm 79	1.68	0.01	1.65	0.03	1	0.005	0.0045	1.2890	0.273	no
Jp 50	1.29	0.01	1.33	0.02	1	0.006	0.0061	4.0580	0.061	no
Jp 51	0.90	0.01	0.90	0.01	1	1.776 E-015	1.776 E-015	1.393 E-012	1.000	no
Ms 31	1.55	0.03	1.60	0.03	1	0.011	0.0105	1.3290	0.266	no
Ms 32	0.65	0.01	0.64	0.04	1	0.001	0.0011	0.1220	0.732	no
Ms 33	0.77	0.02	0.68	0.01	1	0.039	0.0385	22.1230	< 0.001	yes
Ms 34	1.19	0.01	1.13	0.01	1	0.020	0.0195	51.7210	< 0.001	yes
Ms 35	1.07	0.01	1.14	0.01	1	0.027	0.0265	34.7650	< 0.001	yes

Appendix C

C. 2. Summary of Statistical Analysis (one-way ANOVA)

strain	mean colony extension rate				DF	SS	MS	F	P	
	MEAS	SEM	PDAS	SEM						
Ms 36	1.59	0.01	1.70	0.01	1	0.061	0.0613	51.2200	< 0.001	yes
Ms 37	0.90	0.02	1.00	0.04	1	0.044	0.0435	5.4630	0.030	yes
Ms 38	1.20	0.00	1.19	0.01	1	0.000	0.0005	1.1690	0.296	no
Ns 06	1.02	0.01	1.02	0.01	1	0.000	0.0001	0.0913	0.766	no
Ns 08	0.89	0.01	0.93	0.01	1	0.010	0.0098	11.9390	0.003	yes
Ns 10	0.95	0.20	0.38	0.01	1	1.492	1.4920	9.1740	0.008	yes
Ns 11	1.11	0.01	1.06	0.01	1	0.012	0.0116	19.9770	< 0.001	yes
Ns 13	0.68	0.01	0.50	0.02	1	0.161	0.1610	66.5840	< 0.001	yes
Ns 16	0.46	0.01	0.41	0.01	1	0.012	0.0124	12.9820	0.002	yes
Ns 17	1.07	0.01	0.55	0.02	1	1.217	1.2170	507.0000	< 0.001	yes
Ns 18	1.09	0.01	1.08	0.03	1	0.000	0.0000	0.0085	0.928	no
Ns 19	0.59	0.01	0.51	0.01	1	0.031	0.0313	41.4820	< 0.001	yes
Ns 20	1.13	0.01	1.16	0.01	1	0.003	0.0026	4.5940	0.048	yes
Ns 21	0.61	0.01	0.51	0.01	1	0.044	0.0435	68.8850	< 0.001	yes
Ns 29	0.73	0.04	0.57	0.05	1	0.118	0.1180	7.4920	0.015	yes
Ns 30	0.84	0.04	0.55	0.01	1	0.372	0.3720	43.7310	< 0.001	yes
Ns 45	0.90	0.06	0.76	0.04	1	0.079	0.0790	4.0670	0.061	no
Uk 80	0.99	0.01	1.04	0.00	1	0.011	0.0113	48.3870	< 0.001	yes
Uk 81	0.82	0.02	0.71	0.03	1	0.047	0.0473	8.2740	0.011	yes
Uk 82	0.47	0.01	0.42	0.04	1	0.009	0.0095	1.6360	0.219	no
Uk 83	1.09	0.01	1.15	0.01	1	0.016	0.0158	21.6660	< 0.001	yes
Uk 84	0.41	0.01	0.28	0.01	1	0.073	0.0732	61.1950	< 0.001	yes
Uk 85	1.13	0.01	1.20	0.01	1	0.019	0.0190	44.8230	< 0.001	yes
Uk 86	1.06	0.00	0.98	0.03	1	0.027	0.0265	7.9950	0.012	yes
Uk 88	0.84	0.01	0.79	0.01	1	0.012	0.0124	9.9190	0.006	yes
Uk 90	0.66	0.04	0.53	0.02	1	0.085	0.0851	10.1880	0.006	yes
Uk 92	1.07	0.01	1.08	0.01	1	0.001	0.0013	2.0950	0.167	no

Appendix D

Carbon utilization profiles of the clusters of *D. arenaria* and *D. salina*

D. 1. Summary of Statistical Anaylsis (BIOLOG Phenotype Microarrays)

substrates (C sources)	mycelial growth (mean turbidity data ± SEM at 750 nm)				statistical analysis (one-way ANOVA)					
	D. arenaria cluster 1 (n = 15)	***D. salina*** cluster 2 (n = 27)	***D. arenaria*** cluster 3A (n = 5)	***D. salina*** cluster 3B (n = 5)	**DF**	**SS**	**MS**	**F**	**P**	
turanose	**0.952 ± 0.02**	0.628 ± 0.01	0.689 ± 0.04	0.863 ± 0.03	3	1.0970	0.3660	89.401	< 0.001	yes
D-mannose	**0.939 ± 0.03**	0.440 ± 0.01	0.682 ± 0.04	0.768 ± 0.05	3	2.5120	0.8370	111.506	< 0.001	yes
succinamic acid	**0.814 ± 0.02**	0.611 ± 0.01	0.622 ± 0.02	0.782 ± 0.01	3	0.4660	0.1550	28.687	< 0.001	yes
2-keto-D-gluconic acid	0.884 ± 0.03	0.794 ± 0.02	0.821 ± 0.07	**0.984 ± 0.04**	3	0.1910	0.0637	6.620	< 0.001	yes
Quinic acid	**0.743 ± 0.06**	0.156 ± 0.01	0.710 ± 0.07	0.218 ± 0.02	3	4.0110	1.3370	64.926	< 0.001	yes
L-alanine	**0.953 ± 0.04**	0.638 ± 0.02	0.703 ± 0.02	0.795 ± 0.06	3	0.9780	0.3260	21.558	< 0.001	yes
α-D-lactose	**0.823 ± 0.02**	0.465 ± 0.01	0.722 ± 0.04	0.690 ± 0.05	3	1.3440	0.4480	87.975	< 0.001	yes
lactulose	**0.808 ± 0.02**	0.441 ± 0.01	0.668 ± 0.03	0.568 ± 0.03	3	1.3400	0.4470	109.426	< 0.001	yes
β-methyl-D-galactoside	**0.792 ± 0.03**	0.472 ± 0.01	0.667 ± 0.02	0.698 ± 0.03	3	1.0650	0.3550	66.660	< 0.001	yes
maltotriose	**0.784 ± 0.02**	0.450 ± 0.01	0.537 ± 0.03	0.731 ± 0.05	3	1.2060	0.4020	83.978	< 0.001	yes
D-cellobiose	**0.780 ± 0.02**	0.452 ± 0.01	0.657 ± 0.03	0.710 ± 0.03	3	1.1500	0.3830	83.918	< 0.001	yes
sucrose	**0.822 ± 0.03**	0.460 ± 0.01	0.645 ± 0.02	0.801 ± 0.02	3	1.4680	0.4890	78.141	< 0.001	yes
D-xylose	**0.762 ± 0.02**	0.431 ± 0.01	0.643 ± 0.03	0.676 ± 0.03	3	1.1610	0.3870	111.152	< 0.001	yes
L-glutamic acid	**0.740 ± 0.02**	0.591 ± 0.02	0.638 ± 0.01	0.689 ± 0.02	3	0.2250	0.0752	11.700	< 0.001	yes
D-melezitose	**0.745 ± 0.03**	0.505 ± 0.01	0.574 ± 0.03	0.714 ± 0.03	3	0.6250	0.2080	34.589	< 0.001	yes

Appendix D

D. 1. Summary of Statistical Anaylsis (BIOLOG Phenotype Microarrays)

substrates (C sources)	mycelial growth (mean turbidity data ± SEM at 750 nm)				statistical analysis (one-way ANOVA)					
	D. arenaria cluster 1 (n = 15)	*D. salina* cluster 2 (n = 27)	*D. arenaria* cluster 3A (n = 5)	*D. salina* cluster 3B (n = 5)	DF	SS	MS	F	P	
xylitol	**0.527 ± 0.05**	0.181 ± 0.00	0.421± 0.06	0.213 ± 0.01	3	1.2650	0.4220	32.039	< 0.001	yes
gentobiose	**0.772 ± 0.02**	0.383 ± 0.01	0.632 ± 0.03	0.677 ± 0.06	3	1.6030	0.5340	118.161	< 0.001	yes
fumaric acid	0.658 ± 0.01	0.626 ± 0.01	0.522 ± 0.01	**0.695 ± 0.02**	3	0.0906	0.0302	15.035	< 0.001	yes
maltose	**0.752 ± 0.03**	0.410 ± 0.01	0.562 ± 0.04	0.711 ± 0.03	3	1.2660	0.4220	83.269	< 0.001	yes
D-fructose	**0.767 ± 0.03**	0.410 ± 0.01	0.620 ± 0.03	0.623 ± 0.06	3	1.2860	0.4290	55.695	< 0.001	yes
D-galactose	**0.679 ± 0.02**	0.361 ± 0.01	0.603 ± 0.01	0.573 ± 0.01	3	1.0790	0.3600	129.079	< 0.001	yes
L-rhamnose	**0.658 ± 0.02**	0.313 ± 0.01	0.462 ± 0.04	0.308 ± 0.06	3	1.2260	0.4090	59.998	< 0.001	yes
m-inositol	**0.595 ± 0.04**	0.291 ± 0.01	0.405 ± 0.02	0.325 ± 0.02	3	0.9190	0.3060	31.975	< 0.001	yes
α-methyl-D-glucoside	0.539 ± 0.03	0.508 ± 0.01	0.411 ± 0.04	**0.594 ± 0.02**	3	0.0949	0.0316	4.969	0.004	yes
L-asparagine	**0.610 ± 0.01**	0.523 ± 0.01	0.529 ± 0.02	0.543 ± 0.02	3	0.0766	0.0255	8.675	< 0.001	yes
α-D-glucose	0.744 ± 0.03	0.450 ± 0.01	0.613 ± 0.04	**0.749 ± 0.03**	3	1.0120	0.3370	52.461	< 0.001	yes
L-serine	**0.687 ± 0.02**	0.549 ± 0.02	0.580 ± 0.04	0.575 ± 0.05	3	0.1890	0.0630	8.679	< 0.001	yes
sebacic acid	**0.551 ± 0.03**	0.448 ± 0.01	0.276 ± 0.02	0.418 ± 0.01	3	0.3070	0.1020	15.899	< 0.001	yes
palatinose	**0.584 ± 0.02**	0.442 ± 0.01	0.435 ± 0.02	0.554 ± 0.03	3	0.2280	0.0761	22.071	< 0.001	yes
succinic acid mono-methyl ester	**0.547 ± 0.01**	0.443 ± 0.02	0.456 ± 0.01	0.348 ± 0.04	3	0.1830	0.0610	8.736	< 0.001	yes
β-methyl-D-glucoside	0.574 ± 0.01	0.414 ± 0.01	0.506 ± 0.04	**0.644 ± 0.04**	3	0.3790	0.1260	44.000	< 0.001	yes
D-raffinose	**0.595 ± 0.02**	0.341 ± 0.00	0.445 ± 0.03	0.445 ± 0.02	3	0.6270	0.2090	73.689	< 0.001	yes

Appendix D

D. 1. Summary of Statistical Anaylsis (BIOLOG Phenotype Microarrays)

substrates (C sources)	mycelial growth (mean turbidity data ± SEM at 750 nm)				statistical analysis (one-way ANOVA)					
	D. arenaria cluster 1 (n = 15)	*D. salina* cluster 2 (n = 27)	*D. arenaria* cluster 3A (n = 5)	*D. salina* cluster 3B (n = 5)	DF	SS	MS	F	P	
L-aspartic acid	0.531 ± 0.01	0.509 ± 0.01	0.450 ± 0.03	**0.561 ± 0.02**	3	0.0367	0.0122	4.139	0.011	yes
L-arabinose	**0.502 ± 0.02**	0.292 ± 0.00	0.461 ± 0.04	0.382 ± 0.01	3	0.4650	0.1550	66.390	< 0.001	yes
L-proline	**0.480 ± 0.01**	0.347 ± 0.00	0.441 ± 0.05	0.465 ± 0.04	3	0.2000	0.0666	26.980	< 0.001	yes
dextrin	**0.581 ± 0.02**	0.352 ± 0.01	0.505 ± 0.01	0.411 ± 0.02	3	0.5310	0.1770	46.049	< 0.001	yes
succinic acid	0.536 ± 0.01	0.529 ± 0.01	0.503 ± 0.02	**0.581 ± 0.02**	3	0.0168	0.0056	3.320	0.027	yes
D-melibiose	**0.544 ± 0.02**	0.364 ± 0.01	0.420 ± 0.03	0.528 ± 0.03	3	0.3570	0.1190	41.112	< 0.001	yes
D-glucuronic acid	**0.501 ± 0.02**	0.397 ± 0.01	0.428 ± 0.04	0.464 ± 0.02	3	0.1090	0.0363	11.246	< 0.001	yes
L-sorbose	**0.511 ± 0.02**	0.377 ± 0.01	0.296 ± 0.02	0.462 ± 0.03	3	0.2600	0.0866	36.073	< 0.001	yes
arbutin	**0.515 ± 0.01**	0.223 ± 0.00	0.511 ± 0.04	0.339 ± 0.01	3	0.9720	0.3240	174.090	< 0.001	yes
L-malic acid	0.540 ± 0.01	0.560 ± 0.01	0.434 ± 0.04	**0.632 ± 0.01**	3	0.1050	0.0349	8.663	< 0.001	yes
D-ribose	**0.439 ± 0.01**	0.241 ± 0.01	0.356 ± 0.03	0.258 ± 0.02	3	0.4050	0.1350	65.026	< 0.001	yes
stachyose	**0.483 ± 0.01**	0.346 ± 0.01	0.385 ± 0.01	0.389 ± 0.02	3	0.1810	0.0603	20.689	< 0.001	yes
D-trehalose	0.446 ± 0.02	0.400 ± 0.01	0.331 ± 0.02	**0.505 ± 0.02**	3	0.0963	0.0321	12.667	< 0.001	yes
L-ornithine	0.394 ± 0.01	0.383 ± 0.01	0.341 ± 0.02	**0.483 ± 0.04**	3	0.0570	0.0190	8.041	< 0.001	yes
α-methyl-D-galactoside	0.404 ± 0.01	0.413 ± 0.01	0.347 ± 0.01	0.431 ± 0.03	3	0.0215	0.0072	2.613	0.062	no
glycyl-L-glutamic acid	0.432 ± 0.02	0.435 ± 0.01	0.368 ± 0.04	**0.537 ± 0.02**	3	0.0740	0.0247	8.040	< 0.001	yes
Glycogen	**0.381 ± 0.02**	0.342 ± 0.00	0.309 ± 0.01	0.356 ± 0.01	3	0.0247	0.0082	5.574	0.002	yes

Appendix D

D. 1. Summary of Statistical Anaylsis (BIOLOG Phenotype Microarrays)

substrates (C sources)	mycelial growth (mean turbidity data ± SEM at 750 nm)				statistical analysis (one-way ANOVA)					
	D. arenaria cluster 1 (n = 15)	*D. salina* cluster 2 (n = 27)	*D. arenaria* cluster 3A (n = 5)	*D. salina* cluster 3B (n = 5)	DF	SS	MS	F	P	
L-alanyl-glycine	**0.493 ± 0.02**	0.394 ± 0.01	0.437 ± 0.04	0.490 ± 0.03	3	0.1110	0.0369	7.928	< 0.001	yes
Tween 80	**0.392 ± 0.01**	0.365 ± 0.00	0.361 ± 0.01	0.389 ± 0.01	3	0.0090	0.0030	5.292	0.003	yes
Adonitol	**0.390 ± 0.01**	0.342 ± 0.00	0.309 ± 0.02	0.369 ± 0.02	3	0.0341	0.0114	7.067	< 0.001	yes
D-arabinose	**0.335 ± 0.01**	0.239 ± 0.00	0.265 ±0.02	0.284 ± 0.02	3	0.0897	0.0299	28.689	< 0.001	yes
bromosuccinic acid	**0.340 ± 0.01**	0.213 ± 0.01	0.330 ± 0.02	0.252 ± 0.02	3	0.1770	0.0591	45.966	< 0.001	yes
Maltitol	0.346 ± 0.02	0.375 ± 0.01	0.296 ± 0.02	**0.416 ± 0.03**	3	0.0451	0.0150	6.446	< 0.001	yes
g-amino-butyric acid	**0.336 ± 0.01**	0.242 ± 0.01	0.333 ± 0.03	0.313 ± 0.01	3	0.1030	0.0344	22.378	< 0.001	yes
L-phenylalanine	0.280 ± 0.01	0.264 ± 0.01	0.297 ± 0.03	**0.325 ± 0.02**	3	0.0183	0.0061	3.866	0.014	yes
D-mannitol	0.305 ± 0.01	0.447 ± 0.01	0.257 ± 0.05	**0.584 ± 0.03**	3	0.4680	0.1560	55.910	< 0.001	yes
D-arabitol	0.322 ± 0.01	0.322 ± 0.00	0.290 ± 0.04	0.330 ± 0.02	3	0.0052	0.0017	1.335	0.274	no
D-gluconic acid	**0.266 ± 0.01**	0.220 ± 0.00	0.239 ± 0.02	0.265 ± 0.01	3	0.0243	0.0081	15.571	< 0.001	yes
Salicin	0.281 ± 0.01	0.263 ± 0.00	**0.342 ± 0.02**	0.312 ± 0.02	3	0.0321	0.0107	13.051	< 0.001	yes
β-cyclodextrin	0.272 ± 0.01	0.246 ± 0.01	0.270 ± 0.01	0.232 ± 0.01	3	0.0101	0.0034	2.317	0.087	no
adenosine-5 '-monophosphate	0.214 ± 0.02	0.202 ± 0.00	0.208 ± 0.02	0.191 ± 0.00	3	0.0025	0.0008	0.340	0.796	no
D-malic acid	0.290 ± 0.02	0.271 ± 0.01	0.268 ± 0.04	0.230 ± 0.01	3	0.0137	0.0046	1.170	0.331	no
α-ketoglutaric acid	0.256 ± 0.01	0.244 ± 0.00	0.250 ± 0.02	0.282 ± 0.01	3	0.0063	0.0021	2.783	0.051	no
i-erythritol	0.226 ± 0.01	0.335 ± 0.01	0.160 ± 0.01	**0.342 ± 0.02**	3	0.2140	0.0713	59.958	< 0.001	yes

Appendix D

D. 1. Summary of Statistical Anaylsis (BIOLOG Phenotype Microarrays)

substrates (C sources)	mycelial growth (mean turbidity data ± SEM at 750 nm)				statistical analysis (one-way ANOVA)					
	D. arenaria cluster 1 (n = 15)	*D. salina* cluster 2 (n = 27)	*D. arenaria* cluster 3A (n = 5)	*D. salina* cluster 3B (n = 5)	DF	SS	MS	F	P	
N-acetyl-D-glucosamine	0.269 ± 0.01	0.150 ± 0.00	**0.286 ± 0.08**	0.155 ± 0.00	3	0.1840	0.0613	20.555	< 0.001	yes
Adenosine	0.249 ± 0.01	0.247 ± 0.01	0.257 ± 0.03	0.301 ± 0.03	3	0.0130	0.0043	1.750	0.169	no
L-lactic acid	0.272 ± 0.03	0.240 ± 0.01	0.226 ± 0.01	0.287 ± 0.01	3	0.0191	0.0064	1.374	0.262	no
g-hydroxybutyric acid	0.218 ± 0.01	0.181 ± 0.00	0.193 ± 0.02	**0.221 ± 0.01**	3	0.0167	0.0056	7.698	< 0.001	yes
L-threonine	0.215 ± 0.01	0.172 ± 0.00	0.197 ± 0.02	**0.231 ± 0.02**	3	0.0267	0.0089	10.494	< 0.001	yes
D-sorbitol	0.223 ± 0.00	0.486 ± 0.01	0.229 ± 0.03	**0.620 ± 0.04**	3	1.0580	0.3530	167.038	< 0.001	yes
glycerol	0.214 ± 0.00	0.190 ± 0.00	0.214 ± 0.00	**0.239 ± 0.01**	3	0.0134	0.0045	21.075	< 0.001	yes
r-hydroxyphenylacetic acid	0.205 ± 0.02	0.170 ± 0.00	0.172 ± 0.02	0.182 ± 0.01	3	0.0123	0.0041	1.768	0.166	no
amygdalin	0.177 ± 0.01	0.123 ± 0.00	**0.186 ± 0.02**	0.174 ± 0.01	3	0.0405	0.0135	24.218	< 0.001	yes
β-hydroxybutyric acid	**0.195 ± 0.02**	0.152 ± 0.00	0.165 ± 0.00	0.181 ± 0.00	3	0.0188	0.0063	4.549	0.007	yes
L-alaninamide	**0.183 ± 0.00**	0.137 ± 0.00	0.168 ± 0.03	**0.183 ± 0.01**	3	0.0249	0.0083	15.987	< 0.001	yes
α-D-glucose- 1 -phosphate	0.161 ± 0.00	0.141 ± 0.00	**0.252 ± 0.08**	0.211 ± 0.02	3	0.0643	0.0214	7.689	< 0.001	yes
L-fucose	0.166 ± 0.01	0.169 ± 0.00	0.148 ± 0.01	**0.190 ± 0.01**	3	0.0045	0.0015	4.162	0.011	yes
putrescine	0.166 ± 0.00	0.150 ± 0.00	0.156 ± 0.01	**0.171 ± 0.00**	3	0.0035	0.0012	4.503	0.007	yes
D-tagatose	**0.164 ± 0.01**	0.138 ± 0.00	0.140 ± 0.01	0.142 ± 0.01	3	0.0067	0.0022	7.610	< 0.001	yes
α-cyclodextrin	0.156 ± 0.00	0.152 ± 0.00	0.166 ± 0.02	0.168 ± 0.01	3	0.0018	0.0006	1.594	0.203	no
L-pyroglutamic acid	0.150 ± 0.00	0.153 ± 0.00	0.151 ± 0.01	**0.185 ± 0.01**	3	0.0049	0.0016	3.472	0.023	yes

Appendix D

D. 1. Summary of Statistical Anaylsis (BIOLOG Phenotype Microarrays)

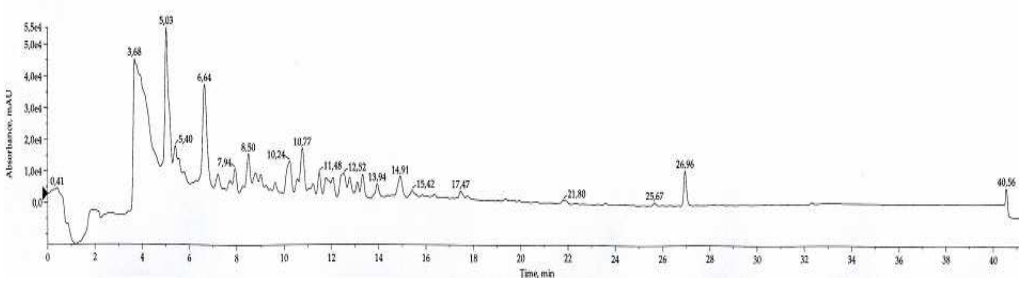
substrates (C sources)	mycelial growth (mean turbidity data ± SEM at 750 nm)				statistical analysis (one-way ANOVA)					
	D. arenaria cluster 1 (n = 15)	*D. salina* cluster 2 (n = 27)	*D. arenaria* cluster 3A (n = 5)	*D. salina* cluster 3B (n = 5)	DF	SS	MS	F	P	
D-psicose	0.162 ± 0.00	0.157 ± 0.00	0.146 ± 0.00	0.151 ± 0.01	3	0.0012	0.0004	1.224	0.311	no
D-glucosamine	**0.148 ± 0.00**	0.109 ± 0.00	0.134 ± 0.01	0.116 ± 0.00	3	0.0160	0.0054	36.196	< 0.001	yes
D-lactic acid methyl ester	0.171 ± 0.02	0.144 ± 0.00	0.139 ± 0.01	0.179 ± 0.01	3	0.0113	0.0038	1.734	0.173	no
D-galacturonic acid	0.116 ± 0.01	0.104 ± 0.00	0.140 ± 0.04	0.101 ± 0.00	3	0.0067	0.0022	2.167	0.104	no
uridine	0.147 ± 0.00	0.154 ± 0.00	0.144 ± 0.01	0.191 ± 0.02	3	0.0082	0.0027	3.919	0.014	yes
N-acetyl-L-glutamic acid	0.135 ± 0.00	0.142 ± 0.00	0.132 ± 0.00	**0.150 ± 0.00**	3	0.0013	0.0004	4.404	0.008	yes
water	0.130 ± 0.00	0.143 ± 0.00	0.145 ± 0.01	**0.154 ± 0.00**	3	0.0030	0.0010	2.826	0.048	yes
2-aminoethanol	0.133 ± 0.00	0.138 ± 0.00	0.123 ± 0.01	**0.167 ± 0.01**	3	0.0056	0.0019	10.599	< 0.001	yes
sedoheptulosan	0.131 ± 0.00	0.137 ± 0.00	0.119 ± 0.01	**0.153 ± 0.01**	3	0.0032	0.0011	7.753	< 0.001	yes
N-acetyl-β- D-mannosamine	0.122 ± 0.00	0.129 ± 0.00	0.124 ± 0.01	0.130 ± 0.00	3	0.0005	0.0002	1.691	0.181	no
N-acetyl-D-galactosamine	0.122 ± 0.00	0.133 ± 0.00	0.121 ± 0.00	**0.134 ± 0.00**	3	0.0016	0.0005	6.774	< 0.001	yes
D-saccharic acid	0.119 ± 0.00	**0.132 ± 0.00**	0.119 ± 0.01	0.151 ± 0.00	3	0.0045	0.0015	11.348	< 0.001	yes
glucuronamide	0.093 ± 0.00	0.094 ± 0.00	0.092 ± 0.01	0.099 ± 0.00	3	0.0002	0.0001	0.554	0.648	no

Appendix E

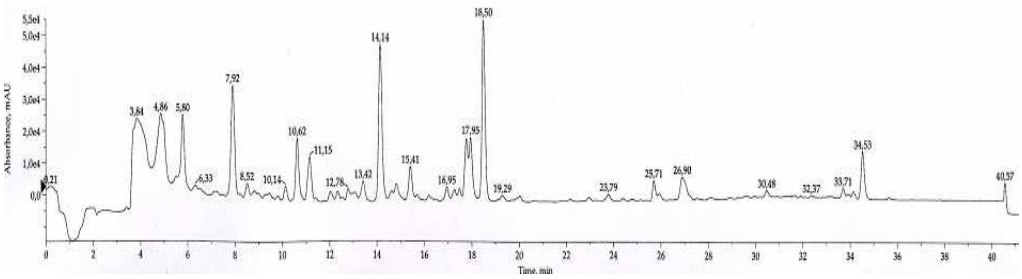
Secondary metabolites and biological activities of the individual *D. arenaria* and *D. salina* strains.

E. 1. High-Performance Liquid Chromatography: Chromatograms

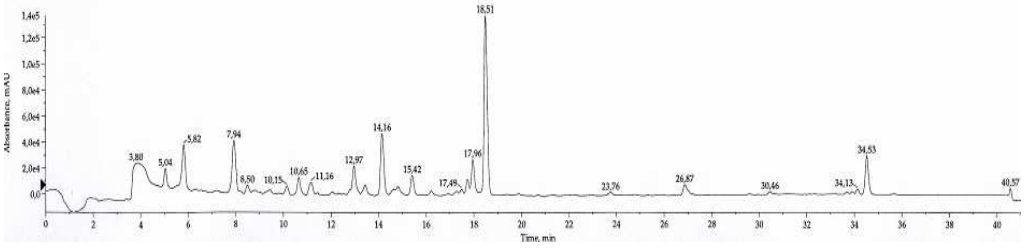
1. MPYS control



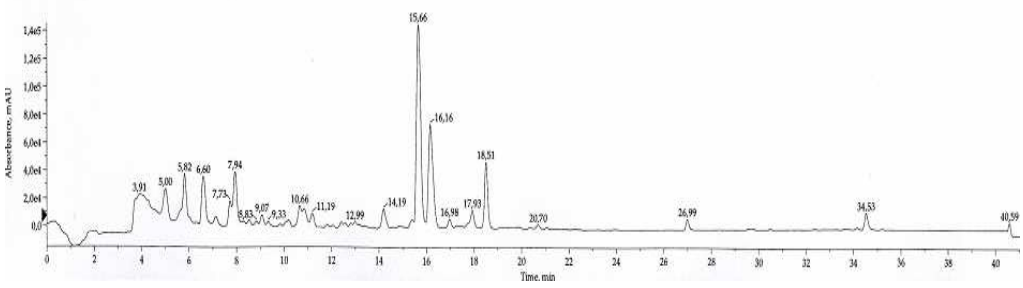
2. *D. arenaria* Bs 02



3. *D. arenaria* Bs 04



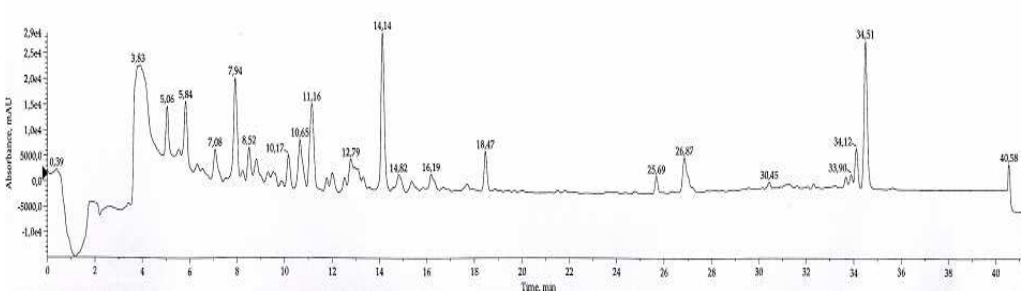
4. *D. salina* Bs 05



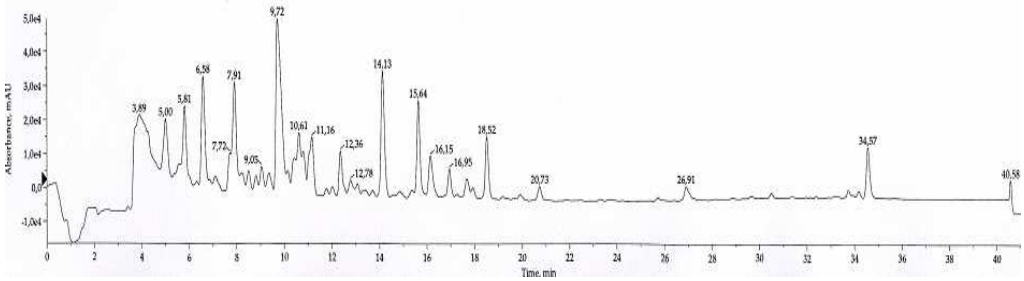
Appendix E

E. 1. High-Performance Liquid Chromatography: Chromatograms

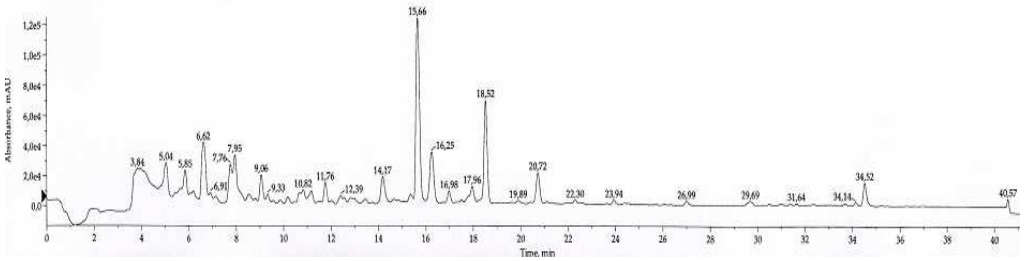
5. *D. arenaria* Bs 94



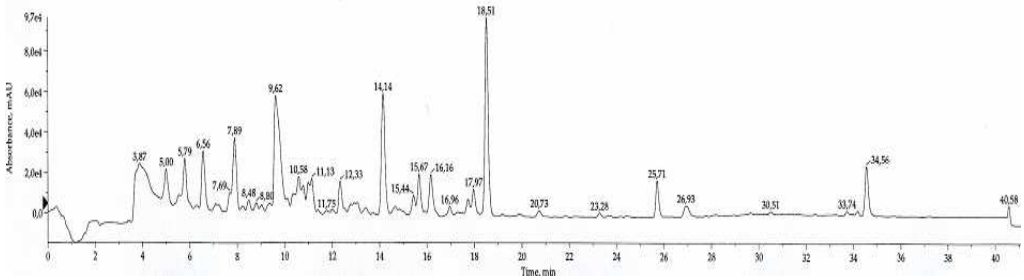
6. *D. salina* Ns 10



7. *D. salina* Ns 21



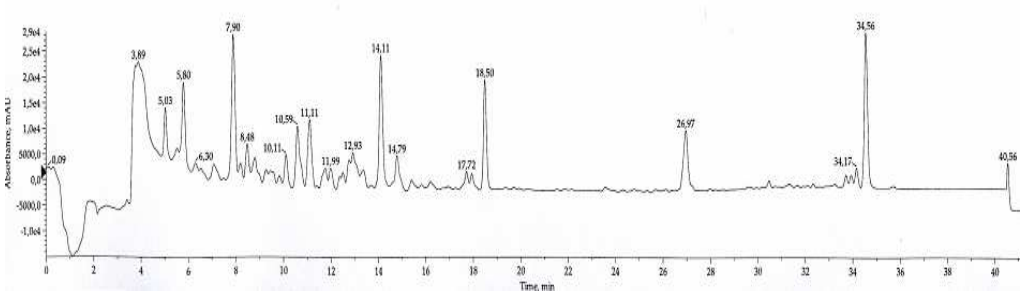
8. *D. salina* Ns 29



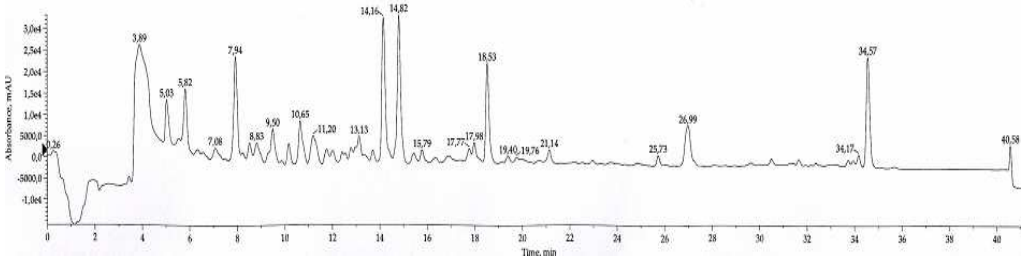
Appendix E

E. 1. High-Performance Liquid Chromatography: Chromatograms

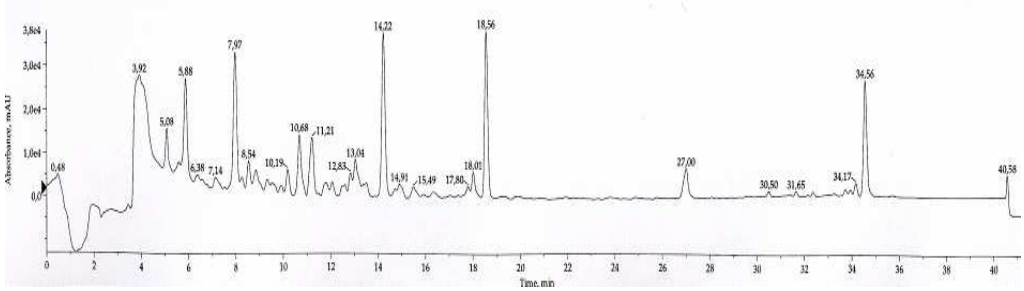
9. *D. arenaria* Gm 22



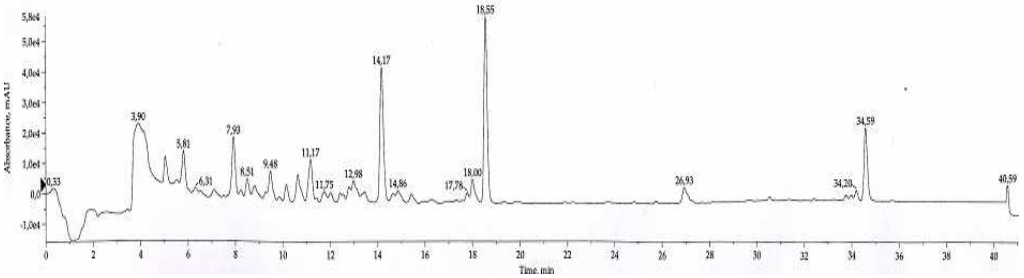
10. *D. arenaria* Gm 23



11. *D. arenaria* Gm 26



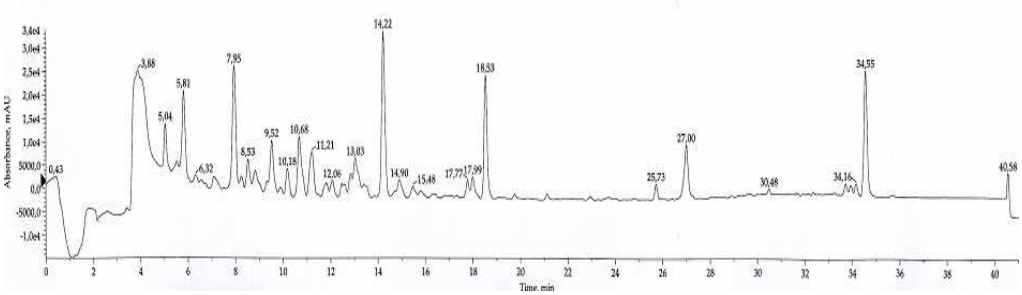
12. *D. arenaria* Gm 56



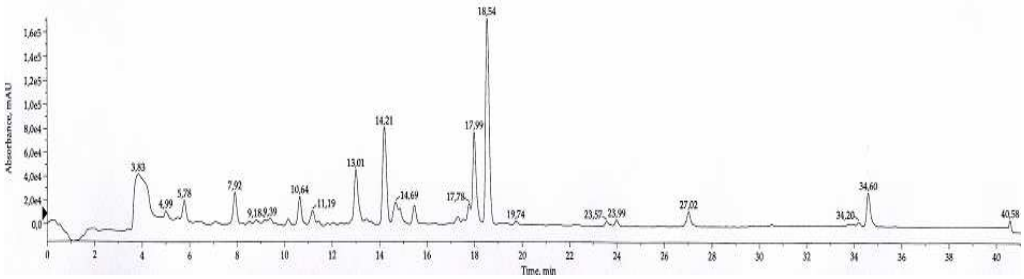
Appendix E

E. 1. High-Performance Liquid Chromatography: Chromatograms

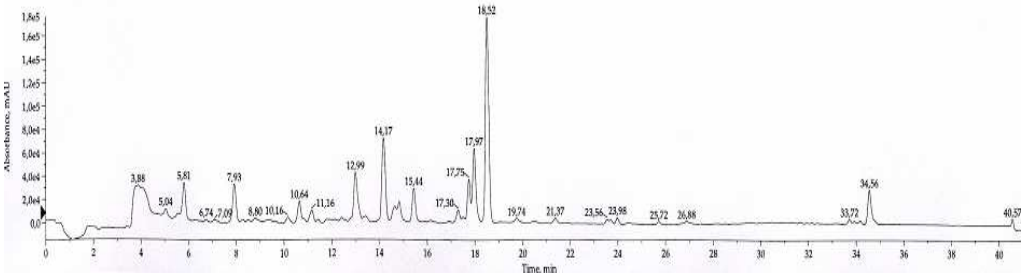
13. *D. arenaria* Gm 79



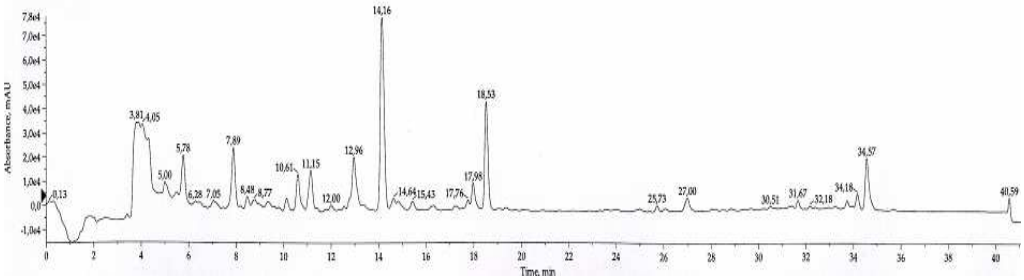
14. *D. arenaria* Jp 50



15. *D. salina* Jp 51



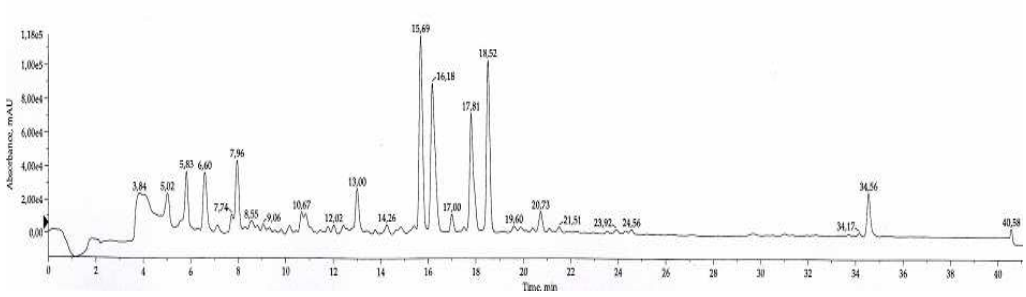
16. *D. arenaria* Fr 52



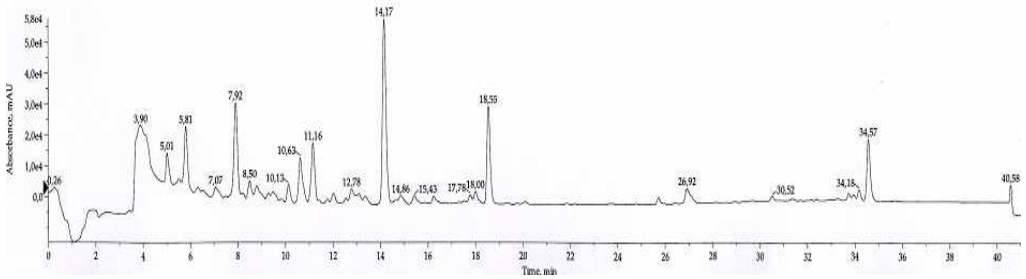
Appendix E

E. 1. High-Performance Liquid Chromatography: Chromatograms

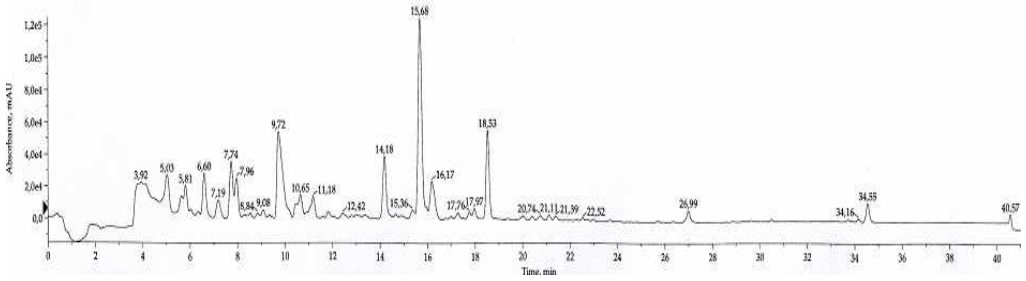
17. *D. salina* Fr 53



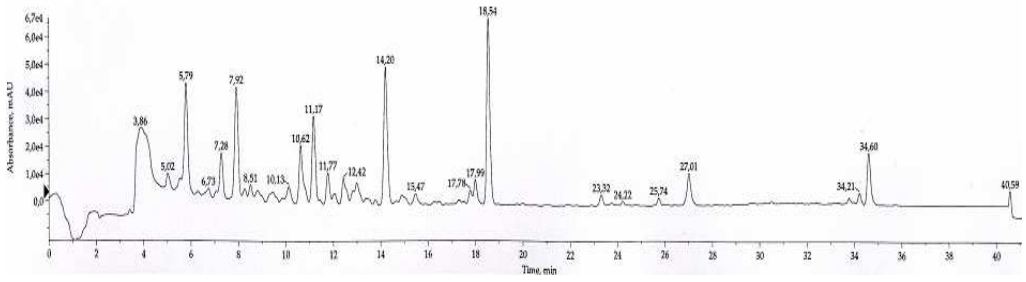
18. *D. arenaria* Fr 54



19. *D. salina* Ms 32



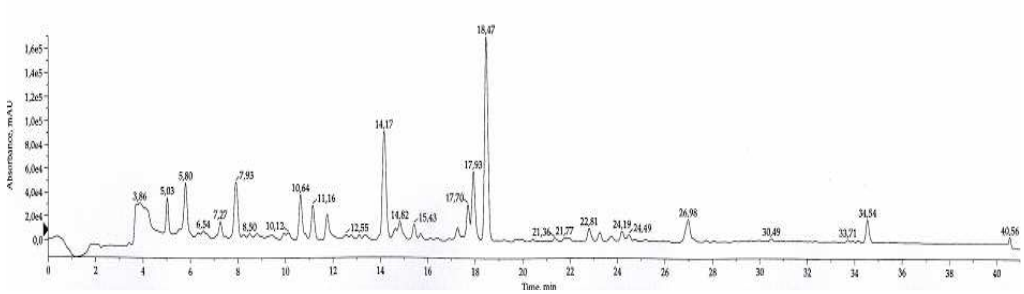
20. *D. salina* Ms 35



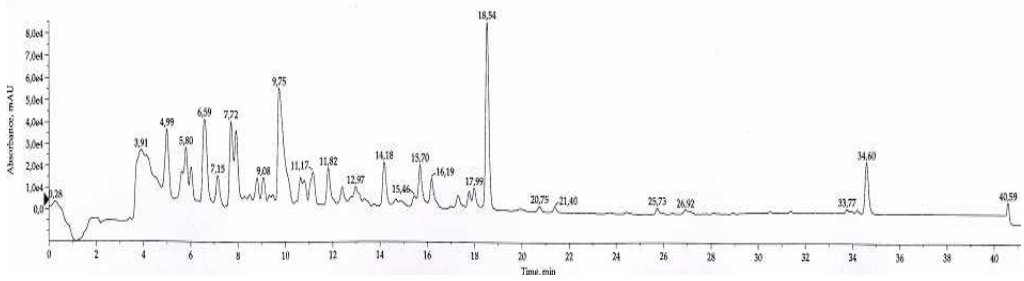
Appendix E

E. 1. High-Performance Liquid Chromatography: Chromatograms

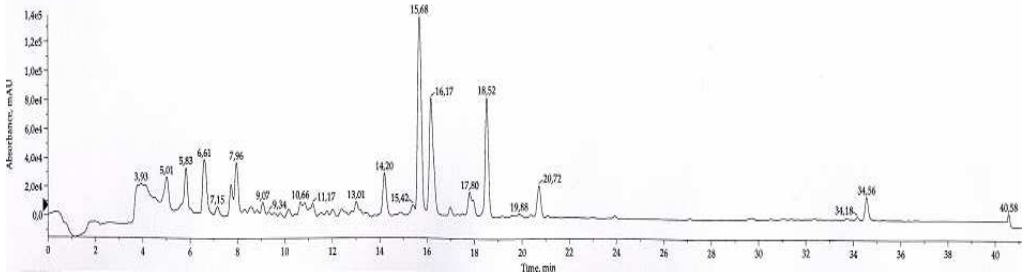
21. *D. salina* Ms 37



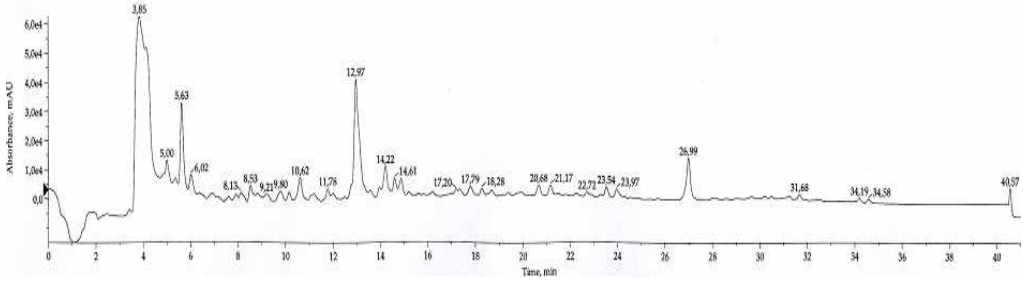
22. *D. salina* Uk 82



23. *D. salina* Uk 90



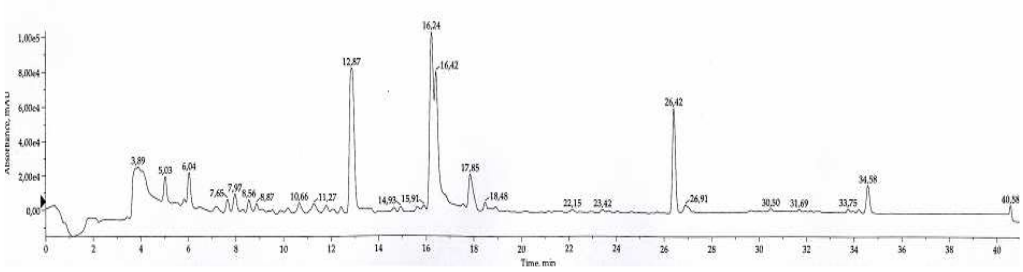
24. *D. vinosa*



Appendix E

E. 3. High-Performance Liquid Chromatography: Chromatograms

25. *Dendryphiella* sp.



Appendix E
E.2. Biological activities of culture crude extracts of *D. arenaria* and *D. salina*

Test Organism: *Microbotryum violaceum*

species	strain	zone of inhibition (in mm)								
		CG	CS	CM	CN	CP	CY	MEA	MPY	PDA
D. arenaria	Bs 7888	0	0	nd	6	7	0	2	nd	0
	Bs 7889	1	0	nd	6	6	0	nd	0	6
	Bs 7891	0	0	nd	5	5	0	0	0	0
	Gm 7541	0	0	nd	5	4	0	0	0	0
	Gm 7479	0	0	0	0	0	0	0	0	0
	Ms 7912	0	0	0	6	9	1	0	0	0
	Ms 7914	0	0	0	6	8	0	0	0	0
	Ms 7916	0	0	0	8	9	1	0	0	0
	Ms 7918	0	0	0	4	10	0	0	0	0
D. salina	Bs 7892	7	6	nd	22	16	13	19	16	10
	Ns 7893	0	0	0	1	4	6	0	nd	nd
	Ns 7897	35	31	27	1	21	25	29	8	10
	Ns 7898	34	30	19	13	6	6	1	1	0
	Ns 7903	31	26	31	32	22	29	30	34	9
	Ns 7909	nd	nd	24	18	25	36	46	12	21
	Gm 7508	0	0	nd	0	0	0	0	0	0

0	no inhibitory activities	20 – 30 mm.	moderately inhibitory activities	nd	no data
< 20 mm.	weakly inhibitory activities	> 30 mm.	strongly inhibitory activities		

Appendix E
E.2. Biological activities of culture crude extracts of *D. arenaria* and *D. salina*

Test Organism: *Chlorella fusca*

species	strain	zone of inhibition (in mm)								
		CG	CS	CM	CN	CP	CY	MEA	MPY	PDA
D. arenaria	Bs 7888	0	0	0	6	4	5	4	nd	3
	Bs 7889	0	0	0	0	4	4	nd	0	5
	Bs 7891	0	0	0	8	12	5	8	0	4
	Gm 7541	0	0	0	5	16	10	5	3	2
	Gm 7479	0	0	0	12	13	10	11	0	4
	Ms 7912	0	0	0	12	14	12	8	6	6
	Ms 7914	0	0	0	9	10	9	8	1	4
	Ms 7916	4	4	0	10	12	4	6	0	4
	Ms 7918	4	0	0	0	13	0	7	6	12
D. salina	Bs 7892	0	0	0	9	14	6	6	0	5
	Ns 7893	0	0	0	9	6	5	5	3	5
	Ns 7897	0	0	0	6	12	12	9	0	0
	Ns 7898	0	0	1	0	12	6	5	0	1
	Ns 7903	0	0	0	9	14	8	6	0	2
	Ns 7909	0	12	0	8	17	10	14	0	6
	Gm 7508	4	0	5	8	12	14	6	2	8

0	no inhibitory activities	20 – 30 mm.	moderately inhibitory activities	nd	no data
< 20 mm.	weakly inhibitory activities	> 30 mm.	strongly inhibitory activities		

Appendix E
E.2. Biological activities of culture crude extracts of *D. arenaria* and *D. salina*

Test Organism: *Bacillus megaterium*

species	strain	zone of inhibition (in mm)								
		CG	CS	CM	CN	CP	CY	MEA	MPY	PDA
D. arenaria	Bs 7888	nd	0	21	7	8	12	15	nd	9
	Bs 7889	6	9	6	8	9	18	10	13	9
	Bs 7891	nd	10	17	8	13	12	7	14	12
	Gm 7541	22	20	21	10	12	15	8	15	11
	Gm 7479	20	17	26	10	13	9	11	15	12
	Ms 7912	16	20	24	12	12	21	10	17	12
	Ms 7914	21	23	24	7	16	17	20	20	16
	Ms 7916	16	17	24	12	11	13	17	12	12
	Ms 7918	19	21	24	11	16	14	17	17	16
D. salina	Bs 7892	nd	nd	nd	10	9	9	12	nd	13
	Ns 7893	19	21	17	9	16	11	11	12	7
	Ns 7897	18	17	nd	12	10	8	10	20	11
	Ns 7898	14	21	12	6	7	10	12	16	13
	Ns 7903	16	18	22	10	10	11	10	nd	12
	Ns 7909	21	28	17	10	8	9	15	12	15
	Gm 7508	25	25	20	7	16	15	11	18	12

0	no inhibitory activities	20 – 30 mm.	moderately inhibitory activities	nd	no data
< 20 mm.	weakly inhibitory activities	> 30 mm.	strongly inhibitory activities		

Appendix E
E.2. Biological activities of culture crude extracts of *D. arenaria* and *D. salina*

Test Organism: *Escherichia coli*

species	strain	zone of inhibition (in mm)								
		CG	CS	CM	CN	CP	CY	MEA	MPY	PDA
D. arenaria	Bs 7888	0	0	0	0	0	0	0	nd	0
	Bs 7889	0	0	0	0	0	0	0	0	0
	Bs 7891	nd	nd	nd	0	0	0	0	0	0
	Gm 7541	0	0	0	0	0	0	0	0	0
	Gm 7479	0	0	0	0	0	0	0	0	0
	Ms 7912	0	0	0	0	0	0	0	0	0
	Ms 7914	0	0	0	0	0	0	0	0	0
	Ms 7916	0	0	0	0	0	0	0	0	0
	Ms 7918	0	0	0	0	0	0	0	0	0
D. salina	Bs 7892	0	0	0	0	0	0	2	0	0
	Ns 7893	0	0	0	0	0	0	0	0	0
	Ns 7897	0	0	nd	0	0	0	0	0	0
	Ns 7898	0	0	nd	0	0	0	0	0	0
	Ns 7903	0	0	0	0	0	0	0	0	0
	Ns 7909	0	0	0	0	0	0	0	0	0
	Gm 7508	0	0	0	0	0	0	0	0	0

0 no inhibitory activities
< 20 mm. weakly inhibitory activities
20 – 30 mm. moderately inhibitory activities
> 30 mm. strongly inhibitory activities
nd no data

Appendix E
E.2. Biological activities of culture crude extracts of *D. arenaria* and *D. salina*

Test Organism: *Vibrio alginolyticus*

species	strain	zone of inhibition (in mm)								
		CG	CS	CM	CN	CP	CY	MEA	MPY	PDA
D. arenaria	Bs 7888	0	0	0	22	13	11	0	nd	9
	Bs 7889	0	0	0	16	18	7	0	0	12
	Bs 7891	nd	nd	nd	14	16	12	0	0	0
	Gm 7541	0	0	0	8	10	0	0	0	7
	Gm 7479	0	0	8	0	0	0	0	0	10
	Ms 7912	0	0	0	18	18	6	10	2	8
	Ms 7914	0	0	0	14	14	0	0	0	8
	Ms 7916	0	0	0	20	15	12	0	0	8
	Ms 7918	0	0	0	18	16	7	0	0	7
D. salina	Bs 7892	0	0	0	0	13	14	8	0	11
	Ns 7893	0	0	0	7	15	12	0	0	8
	Ns 7897	0	0	nd	0	10	8	0	0	9
	Ns 7898	0	0	nd	5	14	10	0	0	8
	Ns 7903	0	0	0	20	8	9	6	0	9
	Ns 7909	0	0	8	9	15	12	0	0	8
	Gm 7508	0	0	0	0	0	0	0	0	0

0	no inhibitory activities	20 – 30 mm.	moderately inhibitory activities	nd	no data
< 20 mm.	weakly inhibitory activities	> 30 mm.	strongly inhibitory activities		

Appendix E
E.2. Biological activities of culture crude extracts of *D. arenaria* and *D. salina*

Culture Media: CDM – sucrose at 18 °C

species	strain	zone of inhibition (in mm)				
		M. violaceum	*C. fusca*	*B. megaterium*	*E. coli*	*V. alginolyticus*
D. arenaria	Bs 7888	nd	nd	nd	nd	nd
	Bs 7889	0	7	26	0	0
	Bs 7891	1	7	19	0	0
	Gm 7541	0	6	24	0	0
	Gm 7479	29	6	29	0	24
	Ms 7912	0	6	16	0	0
	Ms 7914	0	6	11	0	0
	Ms 7916	0	0	14	0	0
	Ms 7918	0	6	10	0	0
D. salina	Bs 7892	44	8	25	1	26
	Ns 7893	25	11	22	0	24
	Ns 7897	37	20	26	0	24
	Ns 7898	24	13	20	0	0
	Ns 7903	42	20	24	0	23
	Ns 7909	43	10	23	0	22
	Gm 7508	0	5	20	0	0

0 no inhibitory activities
< 20 mm. weakly inhibitory activities
20 – 30 mm. moderately inhibitory activities
> 30 mm. strongly inhibitory activities
nd no data

Appendix E
E.2. Biological activities of culture crude extracts of *D. arenaria* and *D. salina*

Culture Media: CDM – sucrose at 25 °C

species	strain	zone of inhibition (in mm)				
		M. violaceum	*C. fusca*	*B. megaterium*	*E. coli*	*V. alginolyticus*
D. arenaria	Bs 7888	0	0	0	0	0
	Bs 7889	0	0	9	0	0
	Bs 7891	0	0	10	nd	nd
	Gm 7541	0	0	20	0	0
	Gm 7479	0	0	17	0	0
	Ms 7912	0	0	20	0	0
	Ms 7914	0	0	23	0	0
	Ms 7916	0	4	17	0	0
	Ms 7918	0	0	21	0	0
D. salina	Bs 7892	6	0	nd	0	0
	Ns 7893	0	0	21	0	0
	Ns 7897	31	0	17	0	0
	Ns 7898	30	0	21	0	0
	Ns 7903	26	0	18	0	0
	Ns 7909	nd	12	28	0	0
	Gm 7508	0	0	25	0	0

0 no inhibitory activities
< 20 mm. weakly inhibitory activities
20 – 30 mm. moderately inhibitory activities
> 30 mm. strongly inhibitory activities
nd no data

Appendix E
E.2. Biological activities of culture crude extracts of *D. arenaria* and *D. salina*

Culture Media: CDM – sucrose at 30 °C

species	strain	zone of inhibition (in mm)				
		M. violaceum	*C. fusca*	*B. megaterium*	*E. coli*	*V. alginolyticus*
D. arenaria	Bs 7888	nd	nd	nd	nd	nd
	Bs 7889	0	7	23	0	0
	Bs 7891	0	8	23	0	0
	Gm 7541	0	4	26	0	0
	Gm 7479	0	5	19	0	0
	Ms 7912	0	12	15	0	0
	Ms 7914	0	4	11	0	0
	Ms 7916	0	0	18	0	0
	Ms 7918	0	5	11	0	0
D. salina	Bs 7892	0	14	24	0	0
	Ns 7893	0	6	10	0	0
	Ns 7897	11	14	15	0	0
	Ns 7898	18	17	11	0	0
	Ns 7903	3	14	25	0	0
	Ns 7909	19	13	16	0	0
	Gm 7508	0	5	22	0	0

0 no inhibitory activities
< 20 mm. weakly inhibitory activities
20 – 30 mm. moderately inhibitory activities
> 30 mm. strongly inhibitory activities
nd no data

Appendix F

Physiological responses of the individual marine *Dendryphiella* strains to abiotic and biotic factors

F. 1. Summary of Statistical Analysis (one-way ANOVA) – salinity

- between different salt concentrations (0, 15, 33 or 45 g L^{-1})

within strain		DF	SS	MS	F	P	
TUBs 7888	Bs 01	3	0.818	0.273	400.845	< 0.001	yes
TUBs 7889	Bs 02	3	1.207	0.402	291.944	< 0.001	yes
TUBS 7891	Bs 04	3	1.432	0.477	346.246	< 0.001	yes
TUBs 7892	Bs 05	3	0.224	0.075	196.538	< 0.001	yes
TUBs 7893	Ns 06	3	1.776	0.592	2463.536	< 0.001	yes
TUBs 7897	Ns 10	3	1.468	0.489	170.134	< 0.001	yes
TUBs 7898	Ns 11	3	0.879	0.293	907.343	< 0.001	yes
TUBs 7903	Ns 16	3	1.138	0.379	237.905	< 0.001	yes
TUBs 7479	Gm 24	3	0.138	0.046	38.262	< 0.001	yes
TUBs 7508	Gm 25	3	0.058	0.019	40.061	< 0.001	yes
TUBs 7541	Gm 27	3	2.398	0.799	34.628	< 0.001	yes
TUBs 7909	Ns 29	3	1.346	0.449	253.777	< 0.001	yes
TUBs 7912	Ms 32	3	0.754	0.251	604.287	< 0.001	yes
TUBs 7914	Ms 34	3	1.196	0.399	1249.365	< 0.001	yes
TUBs 7916	Ms 36	3	0.989	0.330	117.035	< 0.001	yes
TUBs 7918	Ms 38	3	1.656	0.552	1716.048	< 0.001	yes

F. 1. Summary of Statistical Analysis (one-way ANOVA) – temperature

- between diff. incubation temperatures (5, 15, 18, 22, 25, 30, 34, 37 °C)

within strain		DF	SS	MS	F	P	
TUBs 7888	Bs 01	7	20.119	2.874	3184.817	< 0.001	yes
TUBs 7889	Bs 02	7	27.768	3.967	2298.991	< 0.001	yes
TUBS 7891	Bs 04	7	29.714	4.245	3633.041	< 0.001	yes
TUBs 7892	Bs 05	7	9.649	1.378	1735.553	< 0.001	yes
TUBs 7893	Ns 06	7	9.660	1.380	1879.724	< 0.001	yes
TUBs 7897	Ns 10	7	5.789	0.827	675.540	< 0.001	yes
TUBs 7898	Ns 11	7	11.719	1.674	2586.552	< 0.001	yes
TUBs 7903	Ns 16	7	5.835	0.834	295.814	< 0.001	yes
TUBs 7479	Gm 24	7	18.728	2.675	4797.016	< 0.001	yes
TUBs 7508	Gm 25	7	9.918	1.417	1466.870	< 0.001	yes
TUBs 7541	Gm 27	7	18.600	2.657	1850.735	< 0.001	yes
TUBs 7909	Ns 29	7	7.188	1.027	1482.891	< 0.001	yes
TUBs 7912	Ms 32	7	12.395	1.771	2916.481	< 0.001	yes
TUBs 7914	Ms 34	7	12.684	1.812	11050.931	< 0.001	yes
TUBs 7916	Ms 36	7	20.095	2.871	1326.536	< 0.001	yes
TUBs 7918	Ms 38	7	13.749	1.964	4587.421	< 0.001	yes

Appendix F

F. 1. Summary of Statistical Analysis (one-way ANOVA) – pH values

- between different pH values (5, 6.5, 7 or 8)

within strain		DF	SS	MS	F	P	
TUBs 7888	Bs 01	3	0.006	0.002	2.266	0.100	no
TUBs 7889	Bs 02	3	0.026	0.009	4.630	0.008	yes
TUBS 7891	Bs 04	3	0.015	0.005	1.848	0.158	no
TUBs 7892	Bs 05	3	0.008	0.003	2.910	0.050	yes
TUBs 7893	Ns 06	3	0.041	0.014	31.365	< 0.001	yes
TUBs 7897	Ns 10	3	0.365	0.122	43.772	< 0.001	yes
TUBs 7898	Ns 11	3	0.013	0.004	15.921	< 0.001	yes
TUBs 7903	Ns 16	3	0.099	0.033	16.708	< 0.001	yes
TUBs 7479	Gm 24	3	0.014	0.005	3.701	0.022	yes
TUBs 7508	Gm 25	3	0.002	0.001	0.553	0.650	no
TUBs 7541	Gm 27	3	0.205	0.068	17.824	< 0.001	yes
TUBs 7909	Ns 29	3	0.011	0.004	4.809	0.007	yes
TUBs 7912	Ms 32	3	0.032	0.011	8.261	< 0.001	yes
TUBs 7914	Ms 34	3	0.034	0.011	25.004	< 0.001	yes
TUBs 7916	Ms 36	3	0.012	0.004	3.263	0.034	yes
TUBs 7918	Ms 38	3	0.053	0.018	35.189	< 0.001	yes

F. 1. Summary of Statistical Analysis (one-way ANOVA)

- between different *Dendryphiella* strains

between strains	DF	SS	MS	F	P	
salinity						
0 g L^{-1} marine salt	15	12.30	0.82	129.44	< 0.001	yes
15 g L^{-1} marine salt	15	11.50	0.77	537.63	< 0.001	yes
33 g L^{-1} marine salt	15	10.05	0.67	483.88	< 0.001	yes
45 g L^{-1} marine salt	15	11.07	0.74	1105.79	< 0.001	yes
temperature						
15 °C	15	0.66	0.04	58.65	< 0.001	yes
18 °C	15	2.95	0.20	134.53	< 0.001	yes
22 °C	15	11.64	0.78	373.69	< 0.001	yes
25 °C	15	10.05	0.67	483.88	< 0.001	yes
30 °C	15	13.52	0.90	752.78	< 0.001	yes
34 °C	15	9.47	0.63	703.90	< 0.001	yes
pH values						
5.0	15	12.07	0.80	616.63	< 0.001	yes
6.5	15	10.50	0.70	514.24	< 0.001	yes
7.0	15	10.74	0.72	635.74	< 0.001	yes
8.0	15	10.30	0.69	390.76	< 0.001	yes

Appendix F

F. 2. Summary of Statistical Analysis (two-way ANOVA) – salinity vs. temperature

strain			DF	SS	MS	F	P	
TUBs 7888	Bs 01	Factor A (temperature)	3	11.432	3.811	6223.382	< 0.001	yes
		Factor B (salinity)	3	4.020	1.340	2188.261	< 0.001	yes
		Factor A x Factor B	9	1.203	0.134	218.247	< 0.001	yes
TUBs 7889	Bs 02	Factor A (temperature)	3	11.761	3.920	2969.164	< 0.001	yes
		Factor B (salinity)	3	10.984	3.661	2772.990	< 0.001	yes
		Factor A x Factor B	9	3.457	0.384	290.923	< 0.001	yes
TUBS 7891	Bs 04	Factor A (temperature)	3	10.036	3.345	3829.932	< 0.001	yes
		Factor B (salinity)	3	9.522	3.174	3633.812	< 0.001	yes
		Factor A x Factor B	9	3.802	0.423	483.836	< 0.001	yes
TUBs 7892	Bs 05	Factor A (temperature)	3	1.951	0.650	721.711	< 0.001	yes
		Factor B (salinity)	3	3.109	1.036	1150.229	< 0.001	yes
		Factor A x Factor B	9	0.490	0.055	60.434	< 0.001	yes
TUBs 7893	Ns 06	Factor A (temperature)	3	4.867	1.622	2329.725	< 0.001	yes
		Factor B (salinity)	3	5.559	1.853	2660.828	< 0.001	yes
		Factor A x Factor B	9	0.557	0.062	88.928	< 0.001	yes
TUBs 7897	Ns 10	Factor A (temperature)	3	1.480	0.493	376.143	< 0.001	yes
		Factor B (salinity)	3	3.004	1.001	763.343	< 0.001	yes
		Factor A x Factor B	9	1.072	0.119	90.798	< 0.001	yes
TUBs 7898	Ns 11	Factor A (temperature)	3	6.744	2.248	4085.611	< 0.001	yes
		Factor B (salinity)	3	3.097	1.032	1876.298	< 0.001	yes
		Factor A x Factor B	9	0.768	0.085	155.129	< 0.001	yes
TUBs 7903	Ns 16	Factor A (temperature)	3	1.065	0.355	391.280	< 0.001	yes
		Factor B (salinity)	3	3.858	1.286	1417.521	< 0.001	yes
		Factor A x Factor B	9	0.744	0.083	91.115	< 0.001	yes

Appendix F

F. 2. Summary of Statistical Analysis (two-way ANOVA)

strain			DF	SS	MS	F	P	
TUBs 7479	Gm 24	Factor A (temperature)	3	6.559	2.186	2654.089	< 0.001	yes
		Factor B (salinity)	3	3.752	1.251	1518.230	< 0.001	yes
		Factor A x Factor B	9	1.781	0.198	240.199	< 0.001	yes
TUBs 7508	Gm 25	Factor A (temperature)	3	5.588	1.863	2844.927	< 0.001	yes
		Factor B (salinity)	3	2.166	0.722	1102.886	< 0.001	yes
		Factor A x Factor B	9	0.896	0.100	152.056	< 0.001	yes
TUBs 7541	Gm 27	Factor A (temperature)	3	3.263	1.088	173.096	< 0.001	yes
		Factor B (salinity)	3	7.398	2.466	392.532	< 0.001	yes
		Factor A x Factor B	9	2.010	0.223	35.547	< 0.001	yes
TUBs 7909	Ns 29	Factor A (temperature)	3	3.694	1.231	1516.695	< 0.001	yes
		Factor B (salinity)	3	2.848	0.949	1165.353	< 0.001	yes
		Factor A x Factor B	9	0.829	0.092	113.410	< 0.001	yes
TUBs 7912	Ms 32	Factor A (temperature)	3	6.400	2.133	1901.392	< 0.001	yes
		Factor B (salinity)	3	4.740	1.580	1408.392	< 0.001	yes
		Factor A x Factor B	9	2.682	0.298	265.580	< 0.001	yes
TUBs 7914	Ms 34	Factor A (temperature)	3	5.395	1.798	5328.018	< 0.001	yes
		Factor B (salinity)	3	4.805	1.602	4745.127	< 0.001	yes
		Factor A x Factor B	9	1.697	0.189	558.484	< 0.001	yes
TUBs 7916	Ms 36	Factor A (temperature)	3	6.920	2.307	1219.401	< 0.001	yes
		Factor B (salinity)	3	6.717	2.239	1183.634	< 0.001	yes
		Factor A x Factor B	9	2.910	0.323	170.950	< 0.001	yes
TUBs 7918	Ms 38	Factor A (temperature)	3	5.563	1.854	5165.201	< 0.001	yes
		Factor B (salinity)	3	5.444	1.815	5054.372	< 0.001	yes
		Factor A x Factor B	9	1.490	0.166	461.079	< 0.001	yes